RESULTS

Chapter I

1. Isolation and identification of the most active isolate of actinomycetes producing antifungal compound.

1.1 Isolation of actinomycete isolate from different soil samples and screening for production of antifungal.

The collected soil samples were sieved through 3 different types with pore sizes 1.0, 0.5 and 0.25mm to remove contaminant materials. The collected soil samples from different localities of Qaluobia Governorate as illustrate in table (2) were then air dried and mixed well with $CaCO_3$ before plating.

A suspension of the soil sample was prepared by shaking 10 g of soil in 100 ml sterile distilled water and shaked for 20 minutes. The soil suspension was then left for 10 minutes for sedimentation. Serial dilutions were prepared to cover the range of 10^{-3} to 10^{-9} using sterile distilled water. 1.0 ml of each dilution was transferred aseptically to the surface of the nutrient medium in petridish and spread eventually on the surface of the medium using a sterile glass spatula. Triplicate sets of dishes were used for each particular dilution. The petri dishes were then incubated for 10 days at 28°C. Colonies of actinomycetes, which were characterized by their sharp round edges sinking in the agar medium, were picked by a sterile sharp inoculating needle. Streaking several times on the nutrient medium and then subcultured on slants of the same medium purified these colonies.

Three hundreds of actinomycetes cultures were isolated by the methods described previously. The isolated cultures were screened for their capacity of producing antifungal compounds.

Thirteen out of 300 actinomycetes isolates were showed very high activity against the tested dermatophytes fungi, namely ***Tricophyton rubrum, Tricophyton mentagrophytes***, and ***Micosporium canis***. As shown in table (3), the isolates 20, 50, 68, 69, 72, 83, 92, 94, 110, 111, 114, 155 and 160 were more active, and the isolate 68 was the highest active against the tested dermatophytes fungi.
The effect of different metabolite concentrations of selected strain (68) on tested dermatophytes fungi ***Tricophyton rubrum, Tricophyton mentagrophytes***, and ***Micosporium canis*** illustrated in fig. (1).

Table 2 . Actinomycetes isolated from different soil samples

NO. of soil sample	Location	Soil type	Cultivated Plant.	Total count of *Streptomycetes*
1	Toukh	Manure	Zea mays	17
2	Kanater	Manure	Zea mays	7
3	Kafr-shoker	Clay loam	Zea mays	6
4	Shebin El- kanater	Clay loam	Zea mays	10
5	Moshtohor-1	Clay loam	Grapes	27
6	Moshtohor-2	Clay loam	Pomegranate	56
7	Kafr-saad-1	Clay loam	Banana	61
8	Kafr-saad-2	Sandy clay	Banana	30
9	Kafr-saad-3	Clay loam	Cabbage	57
10	Kaha	Clay loam	Onion	29

Table (3): Production of antidermatophytes activity from different isolate of actinomycetes at 30°C after10 days of incubation

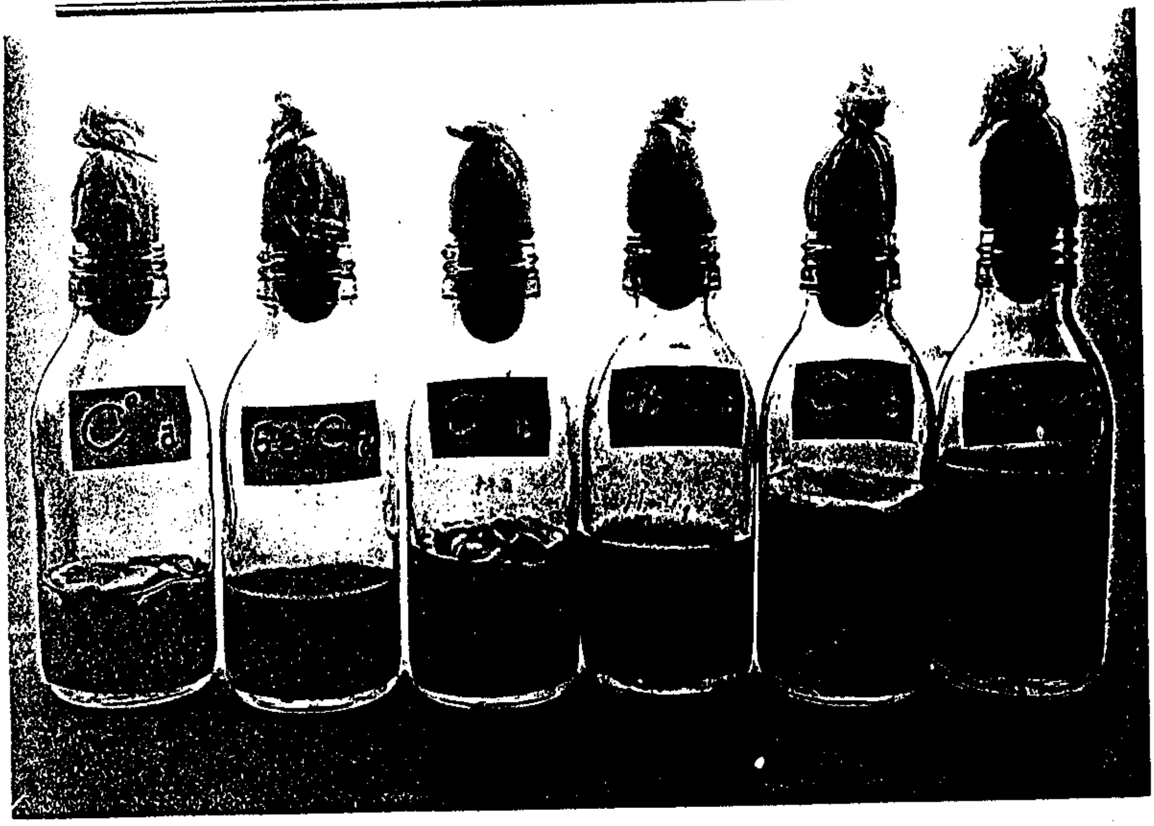
Isolate numder	Growth inhibition rate (%) of *T. rubrum* at different concentration.			pH	Growth inhibition rate (%) of *T. mentagrophytes* at different concentration.			pH	Growth inhibition rate (%) of *M.canis* at different concentration.			pH
	Conc.a	Conc. b	Conc.c		Conc. a	Conc.b	Conc.c		Conc.a	Conc.b	Conc.c	
20	0%	0%	0%	8.5	0%	0%	0%	8	10%	45%	60%	7.8
50	90%	95%	100%	7.5	0%	0%	10%	7.8	33%	53%	75%	8
68	98%	100%	100%	8.5	98%	100%	100%	7.5	100%	100%	100%	8.5
69	96%	100%	100%	9.3	97%	100%	100%	8.5	97%	100%	100%	8.5
72	20%	32%	45%	8	0%	0%	0%	8.5	0%	0%	0%	8.5
83	45%	55%	68%	8.5	0%	0%	0%	8	0%	0%	0%	8
92	0%	0%	0%	8.5	0%	0%	0%	8	98%	100%	100%	9
94	30%	34%	45%	8	0%	0%	0%	8.5	30%	47%	62%	8
110	50%	77%	90%	8	0%	0%	0%	8	0%	0%	0%	7.8
111	85%	90%	95%	9	0%	0%	0%	8	0%	0%	0%	8
114	0%	0%	0%	8.5	0%	0%	0%	8	45%	73%	100%	9
155	0%	0%	0%	8.5	0%	0%	0%	7.7	44%	72%	90%	8.5
160	0%	0%	0%	8.5	0%	0%	0%	8	42%	67%	80%	8
Control	0%	0%	0%	7	0%	0%	0%	7	0%	0%	0%	7

Conc.a= 20 ml of fungal medium + 20 ml of actinomycete filtrate (sterile).
Conc.b= 20 ml of fungal medium + 40 ml of actinomycete filtrate (sterile).
Conc.c= 20 ml of fungal medium + 60 ml of actinomycete filtrate (sterile).

(a)

(b)

Fig.(1): Effect of different concentrations of the isolate No. 68 filtrate on the growth rate of (a) *T. rubrum* (b) *T. mentagrophytes* (c) *M. canis*.



(c)

1.2 Identification of isolate 68 (produces the highest antifungal compound)

The selected isolate was identified to the species level of ***Streptomyces*** as follows:

1.2.1 Diagnostic characteristics and identification of isolate number 68

Investigation of the morphological characteristics of isolate number 68

i- Spore chain morphology: straight (R)(fig. 2)

ii- Spore surface: smooth (S)(fig. 3)

iii- Color of colony: the aerial mycelium in the whitish yellow, pale yellow green, white and yellowish white on oatmeal agar, malt-yeast extract agar, glucose asparagin and fishmeal agar, respectively.

v- Reverse side of colony: distinctive pigments were detected as pale brown on both oatmeal and yeast malt extract but yellow pigment on fishmeal(table 4).

v- Color in media: melanoid pigments were formed on tyrosine agar but were not on peptone yeast iron agar and trypton broth(table 6).

Fig. (2): Micromorphology of spore chains of isolate No. 68 X 1000.

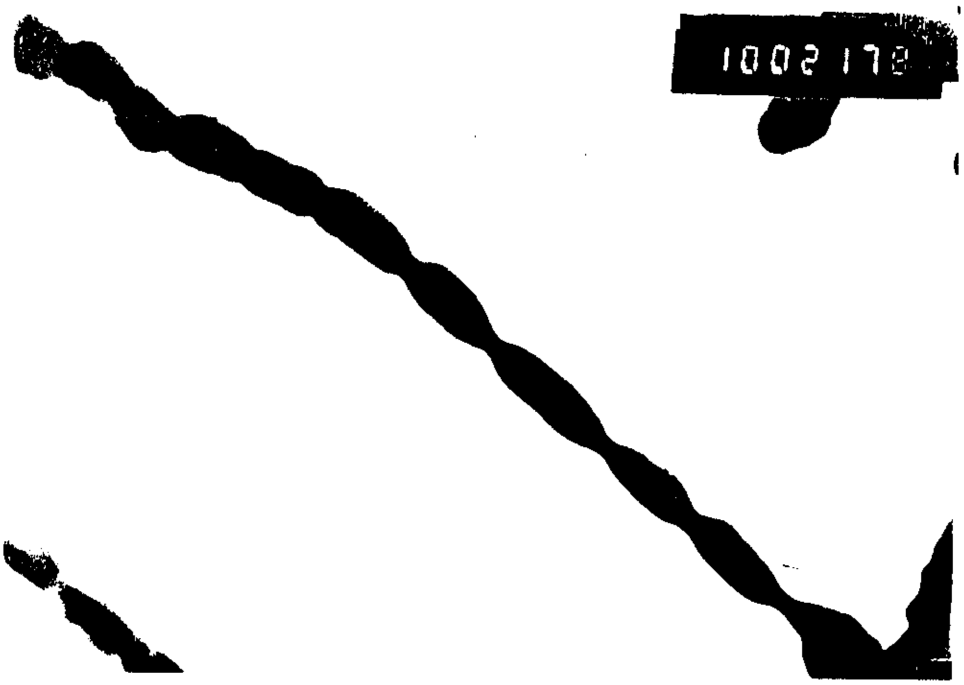


Fig. (3): Electron micrograph of spore surface of isolate o.68

X 40 000.

Table 4 . Morphological and characterization of isolate number 68

Media	Color of			Growth
	Aerial mycelium	Substrate mycelium	Soluble pigment	
Oatmeal agar	Whitish yellow	Pale yellow	Pale brown	+Ve
Malt extract-yeast extract agar	Pale yellow green	Yellow	Pale brown	++++Ve
Inorganic salts-starch agar	Whitish yellow	Yellow	- Ve	+++Ve
Glycerol-asparagine agar	White	White	- Ve	++Ve
Sucrose-nitrate agar	Yellowish white	White	- Ve	+++Ve
Fishmeal agar	Whitish yellow	Yellow	Yellow	+++Ve
Nutrient agar	White	White	- Ve	++Ve
Starch-nitrate agar	Yellow	Brown	- Ve	+++Ve
Glycerol-nitrate agar	White	Whitish yellow	–Ve	+++Ve
Glucose-asparagine agar	White	White	- Ve	++Ve

- Ve = negative = no growth.
+Ve = weak growth.
++Ve = moderate growth.
+++Ve = good growth.
++++Ve = very good growth.

1.2.2 Physiological and biochemical properties of isolate number 68

i-Utilization of carbon sources

As it is clear from the data recorded in table (5) the tested organism 68 succeeds to utilize all carbon sources with different degrees (except inositole, L-rhamnose and glycerol). The starch and mannitol were the best carbon sources that utilized by tested organism (good growth) as illustrated in table (5)

ii-Growth on Czapek's medium

Isolate number 68 showed very good growth on Czapek's medium.

iii-Other physiological and biochemical properties of isolate number 68

From the results in table (6) it is evident that liquefaction of gelatin, starch hydrolysis (amylase), catalase production and reduction of nitrate to nitrite were positive, but hydrogen sulfide production, peptonization and coagulation of skimmed milk and cellulose decomposition were negative.

The isolate also had the ability to breakdown certain complex compounds as L- tyrosine, aesculin, urea, tween 20 and tween 80, but the organism failed to degrade casein table (7).

iv- Growth test

Data of table (8) showed that the isolate 68 grew at 4, 7 and 10% (w/v) of NaCl concentrations but the rate of growth decreased with increasing the concentration till to 14% (w/v) of sodium chloride.

0.1% (w/v) of phenol and 0.001% (w/v) of crystal violet, did not inhibit growth of the tested isolate. Also, the results showed that the tested isolate failed to grow in the presence of 0.01, 0.02 % (w/v) of sodium azide.

v- Resistance to certain antibiotics

From the results recorded in the table (9), the antibiotics vancomycin, rifampicin and penicillin G failed to inhibit the microbial growth, while the antibiotics tobramycin, gentamycin and streptomycin were able to prevent the isolate growth.

Table 5 . Utilization of carbon sources by the isolate 68

Carbon sources	Results
D-Glucose	+Ve
D-Xylose	+Ve
L-Arabinose	+Ve
L-Rhamnose	±Ve
D-Fructose	+Ve
D-Galactose	+Ve
Raffinose	+Ve
D-Mannitol	+Ve
Inositole	−Ve
sucrose	+Ve
Glycerol	±Ve
Maltose	+Ve
Salicin	+Ve
Starch	+++Ve
Lactose	+Ve
Cellulose	+Ve
Cellobiose	+Ve
Control	±Ve

−Ve = No growth

±Ve = doubtful growth

+Ve = Weak growth

+++Ve = Very good growth

Table 6 . **Physiological and biochemical properties of the isolate 68**

Test	Results
Gelatin liquefaction	+Ve
Catalase production	+Ve
Reduction of nitrate	+++Ve
Cellulose decomposition	- Ve
Peptonization & coagulation of milk	- Ve
H_2S production	- Ve
Starch hydrolysis	+Ve
Melanin production	- Ve
Soluble pigment	- Ve

Table 7 . **Other physiological and biochemical properties of isolate 68**

Degradation test	Results
Tyrosine	+Ve
Casein	+Ve
Aesculin	+Ve
Urea	+Ve
Tween 20	+Ve
Tween 80	+Ve

- Ve = Negative result

+ Ve = positive result

Table 8 : Growth rate of isolate 68 in the presence of certain chemical inhibitors

Chemical inhibitor	Results
Sodium chloride conc. (w/v)	
4%	+Ve
7%	+Ve
10%	+Ve
14%	- Ve
Sodium azide conc. (w/v)	
0.01%	±Ve
0.02%	- Ve
Phenol conc. (w/v)	
0.1%	+Ve
Crystal violet conc. (w/v)	
0.001%	+Ve

- Ve = Negative

±Ve = doubtful growth

+Ve = Positive

Table 9 . The ability of isolate 68 to resist certain antibiotic

Antibiotics	Sensitivity
Vancomycin	+Ve
Tobramycin	−Ve
Rifampicin	+Ve
Gentamicin	−Ve
Streptomycin	−Ve
Penicillin G	+Ve

−Ve = No inhibitor (sensitive)

+Ve = Inhibitor (resistance)

Comparing the pervious morphological and physiological characteristics with those of ***Streptomyces*** species included in International Streptomyces Project (I.S.P.) **(Shirling and Gottlieb** 1968a, 1968b, 1969, 1972) and **Bergey (1979& 1994)** and **Szab *et al.*** keys **(1975)** entry as aerial and substrate mycelium color, reverse pigment, the form of sporophore and the source of carbon utilized. These keys lead to the identification of the experimental organism as ***Streptomyces kanamyceticus.***

Waksman key (1961) entry as the form of sporophore in aerial mycelium, melanin pigments negative and weakly proteolytic enzyme, the key lead to the characterization as ***Streptomyces griseoloalbus.***

Descriptions of the *Streptomyces* species given by the various keys

Streptomyces kanamyceticus

Spore chain morphology: straight sporophore with mature spore chains generally 10 to 50 spore per chain, longer chains are sometimes observed on suitable media, this morphology is seen on yeast-malt agar and glycerol asparagin agar, sporulation may be poor on oatmeal agar and salts starch agar.

Spore surface: smooth.

Color of colony: aerial mass color in white to yellow color on glycerol nitrate agar medium.

Melanin pigments production: negative.

Gelatin liquefaction: good liquefaction.

Milk: coagulation, slow peptonization.

Starch: starch hydrolysis.

Antibiotic production: production of kanamycin antibiotic with antifungal activity exhibited by ***Streptomyces*** species.

Na Cl tolerance: tolerance to 7% and hydrolyzate at 10%.

Carbon utilization: D-glucose, L-arabinose, D-xylose, D- fructose, raffinose and D-mannitol are utilized for growth. No growth or only a trace of growth was recorded with inositol, rhamnose or sucrose.

Streptomyces griseoloalbus

Spore chain morphology: growth made up of long straight, profusely branching filaments. Aerial mycelium produces short and straight sporophores. Spores small, oval.

Spore surface: smooth.

Color of colony: aerial mass color in white group becomes dirty grayish-white.

Melanin pigments production: negative.

Gelatin liquefaction: good liquefaction.

Milk: partially peptonization.

Reduction of nitrate to nitrite: positive.

H_2S production: negative.

Starch hydrolysis: positive.

Antibiotic production: producing antifungal grisein (albomycin) yellow group and neomycin complex.

Carbon utilization: D-glucose, D-xylose, L-arabinose, L-rhamnose, D-fructose, D-mannitol, inositol and sucrose are utilized for growth. No growth or only trace of growth was recorded with D- galactose and raffinose.

The description of the above ***Streptomyces*** species were cited from **Shirling and Gottlieb (1969 &1972)** and in **Waksman (1961).**

The present isolate is similar to ***Streptomyces kanamyceticus*** in sporophore form, spore surface, aerial mass colour, reverse pigment, melanoid pigment and carbon utilization as (D-glucose, D-mannitol, L-arabinose, D- xylose, D-fructose and raffinose). On the other hand it differs from ***Streptomyces kanamyceticus*** in that its sporulation heavy when cultivated on malt extract-yeast extract agar. It differs also in carbon utilization for growth sucrose was utilized but rhamnose is doubtful.

From the above mentioned reasons the organism is considered to be a new variety of ***Streptomyces kanamyceticus*** EHE-68.

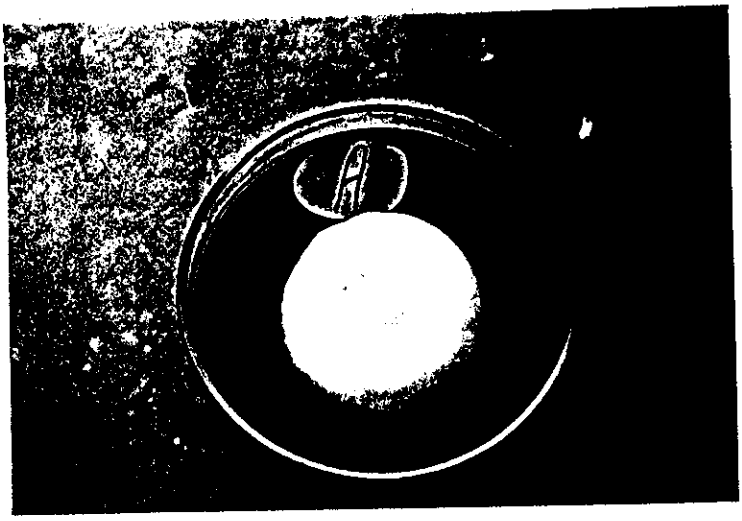
2- Isolation and identification of dermatophyte fungi

Isolation of different isolates of dermatophyte fungi by physicians in dermatology department of Kaha general hospital and Benha University hospital, Qluobia Governorate as illustrate in material and methods. Identification of isolated dermatophyte fungi were illustrated in material and methods and in fig.(4, 5 & 6).

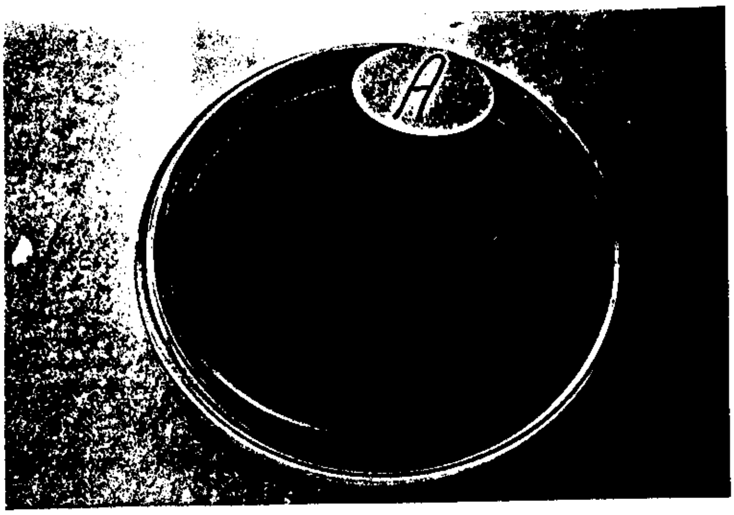
The results in fig.(4 ,a ,b &c) illustrate that the color of growth was white to cream with flat raised growth with win-red reverse color and microscopic examination clear that there is microconidia (clavate to pyriform and macroconidia (smooth, thin walled, cylindrical cigar shape). From these results this isolate named ***T. rubrum.***

The result in fig.(5, a, b & c) illustrate that color of growth was white to cream with powdery to granular flat surface, yellow brown to reddish-brown reverse color and ultra structure illustrate that there is numerous single celled, spherical to subspherical microconidia with spiral hyphae multicelled macroconidia. From these results this isolate named ***T. mentagrophytes.***

The result in fig.(6, a, b & c) illustrate that color of growth was white to cream with spreading to dense cottony flat surface, bright golden-yellow to brown-yellow reverse color and ultra structure illustrate that there is spindle shaped macroconidia with 5-15 cells, which verrucose, thick walled, and they often have a terminal knob. From these results this isolate named ***M. canis.***



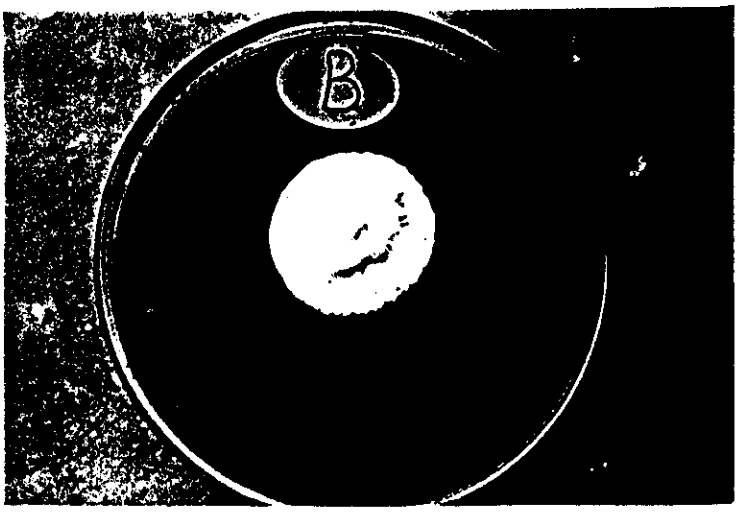
(a)



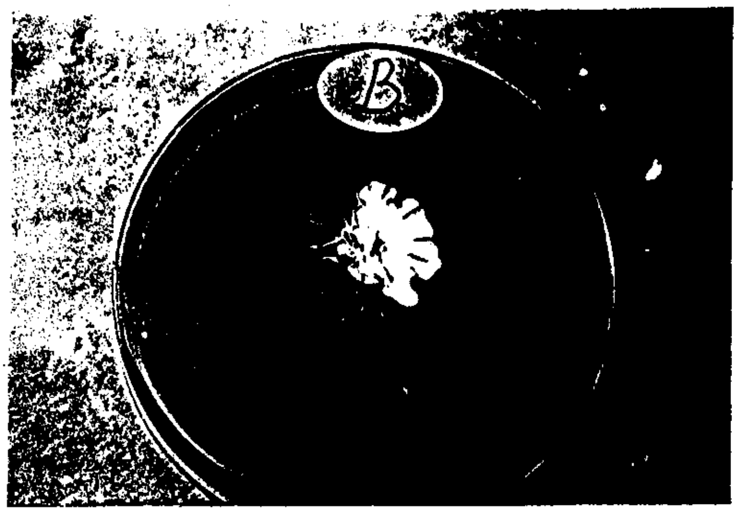
(b)

Fig.(4): Culture of ***T. rubrum*** are (a) Usually flat to slightly raised, white to cream, suede-like to downy, (b) With a win-red reverse color, (c) The microscopic morphology showing numerous numbers of clavate to pyriform microconidia and moderate numbers of smooth thin-walled, cylindrical (cigar) shaped macroconidia.

(c)



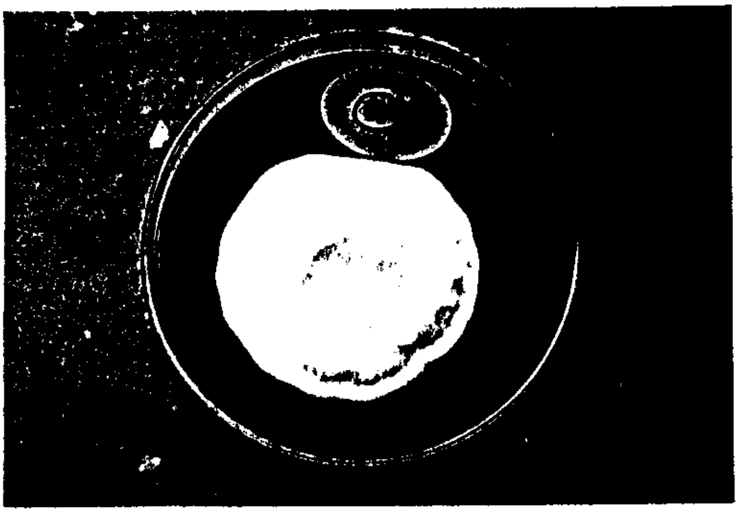
(a)



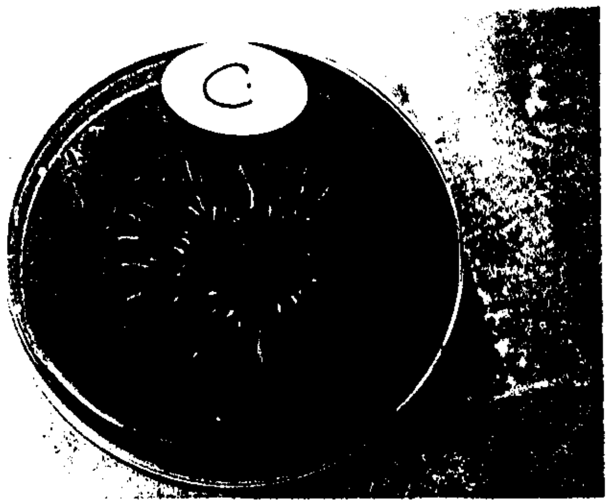
(b)

Fig.(5): Cultures of *T. mentagrophytes* are (a) Generally flate, white to cream in color, with a powdery to granular surface. (b) Yellow brown to reddish- brown reverse color. (c) The microscopic morphology showing numerous singles celled, spherical to subspherical microconidia, often in dense clusters, spiral hyphae, multicelled macroconidia may also be present.

(c)

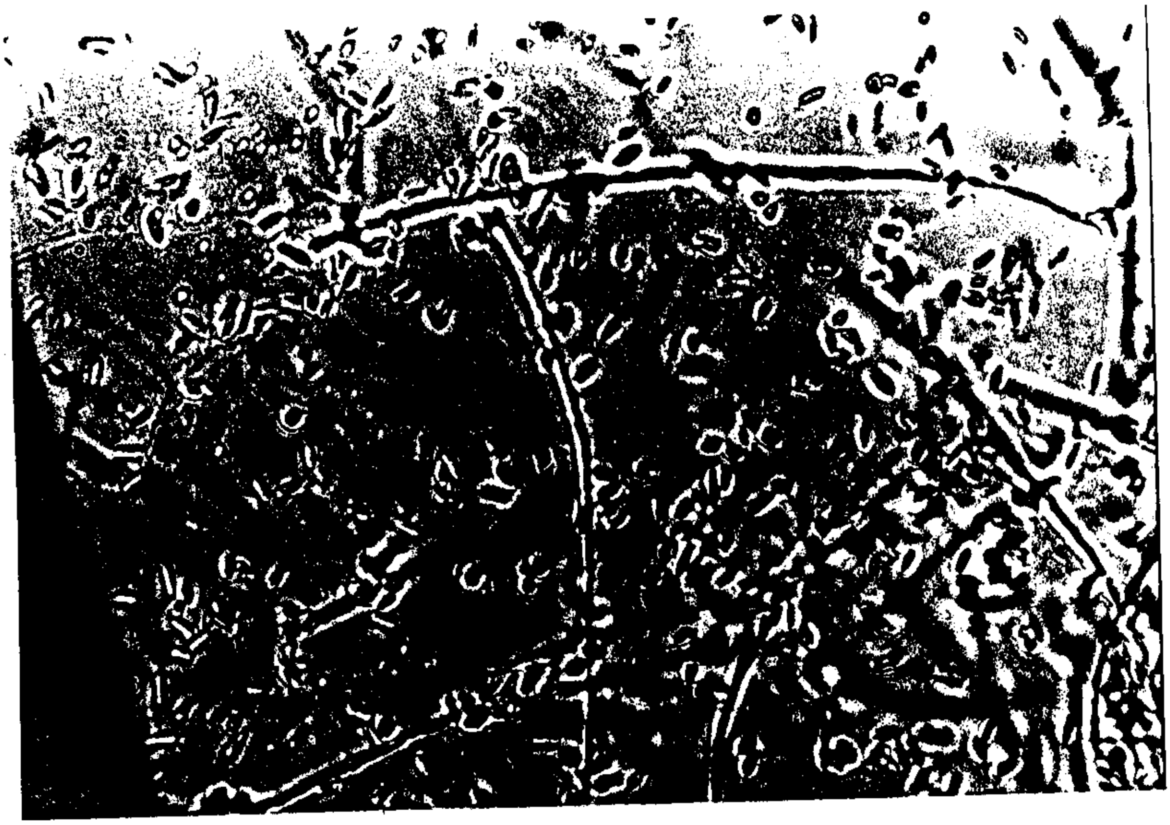


(a)



(b)

Fig.(6): cultures of *M.canis* are (a) Flat, spreading white to cream-colored, with dense cottony surface. (b) They usually have a bright golden-yellow to brown-yellow reverse pigment. (c) Microscopic morphology showing macroconidia are typically spindle shaped with 5-15 cells, verrucose, thick walled, and they often have a terminal knob.



(c)

ChapterII

Factors affecting the antifungal biosynthesis by *Streptomyces kanamyceticus* EHE-68

1. Environmental factors

1.1 Effect of different incubation periods on the extracellular protein, antifungal biosynthesis, growth rate and final pH of *Sterptomyces kanamyceticus* EHE-68

Streptomyces kanamyceticus was cultivated on starch nitrate medium and incubated at 30°C for different incubation times.

This experiment was carried out to elucidate the influence of different incubation periods on growth rate, antifungal production, protein content and final pH of culture filtrate. In this experiment, the culture flasks were incubated at 180 rpm. in an electric shaking incubator and at static condition, also. At the end of each incubation period, the culture was filtrated and centrifuged. The filtrate was then used for estimation of antifungal activity, extracellular protein and final pH.

1.1.1 Shaking condition at 180 rpm.

The obtained results in table (10) and figure (7) showed that any prolong in the incubation period was accompanied by an increase in antifungal activity against ***T. rubrum, T. mentagrophytes*** and ***M. canis*** until a maximum value was obtained after 10 days of incubation. The maximum value of protein was obtained after incubation period for 12 days, then decreased with the prolonged incubation until a minimal value. The results also showed that the values of final pH and growth rate (dry weight g /50ml) were increased with the prolongation of the incubation period, reaching their maximal values at 18 and 10 days, respectively. It is shown from the experiment that the organism needs 10 days of incubation to obtain the maximum value of antidermatophytes produced by ***Streptomyces kanamyceticus*** EHE-68.

1.1.2 Static condition

The results obtained in table (11) and figure (8) showed that the maximum growth obtained after 6 days of incubation. The antifungal activity began to produce by 49, 50 and 52% after 14 days of incubation at ***T. rubrum, T. mentagrophytes*** and ***M. canis,*** receptively. That was increasing antifungal activity with increasing incubation period to reach 60, 62 and 64% at ***T. rubrum, T. mentagrophytes*** and ***M.canis,*** respectively.

Table 10 . Effect of different incubation periods on antifungal biosynthesis, extracellular protein, final pH and growth rate of *Streptomyces kanamyceticus* EHE-68 (under shaking condition at 180 rpm, at 30°C)

Incubation time (days)	Growth (dry weight) (g / 50ml)	Final pH	Protein (mg/ml)	Growth inhibition rate (%) of		
				T.rubrum	*T.mentagrophytes*	*M.canis*
2	0.82	7.3	1.482	0%	0%	0%
4	1.02	7.3	3.221	60%	60%	60%
6	1.40	7.3	3.234	80%	80%	80%
8	1.48	7.3	3.354	80%	80%	80%
10	1.56	8.0	3.530	100%	100%	100%
12	1.08	8.2	3.798	100%	100%	100%
14	0.98	8.5	3.425	100%	100%	100%
16	0.76	8.8	2.868	100%	100%	100%
18	0.62	9.3	2.310	100%	100%	100%

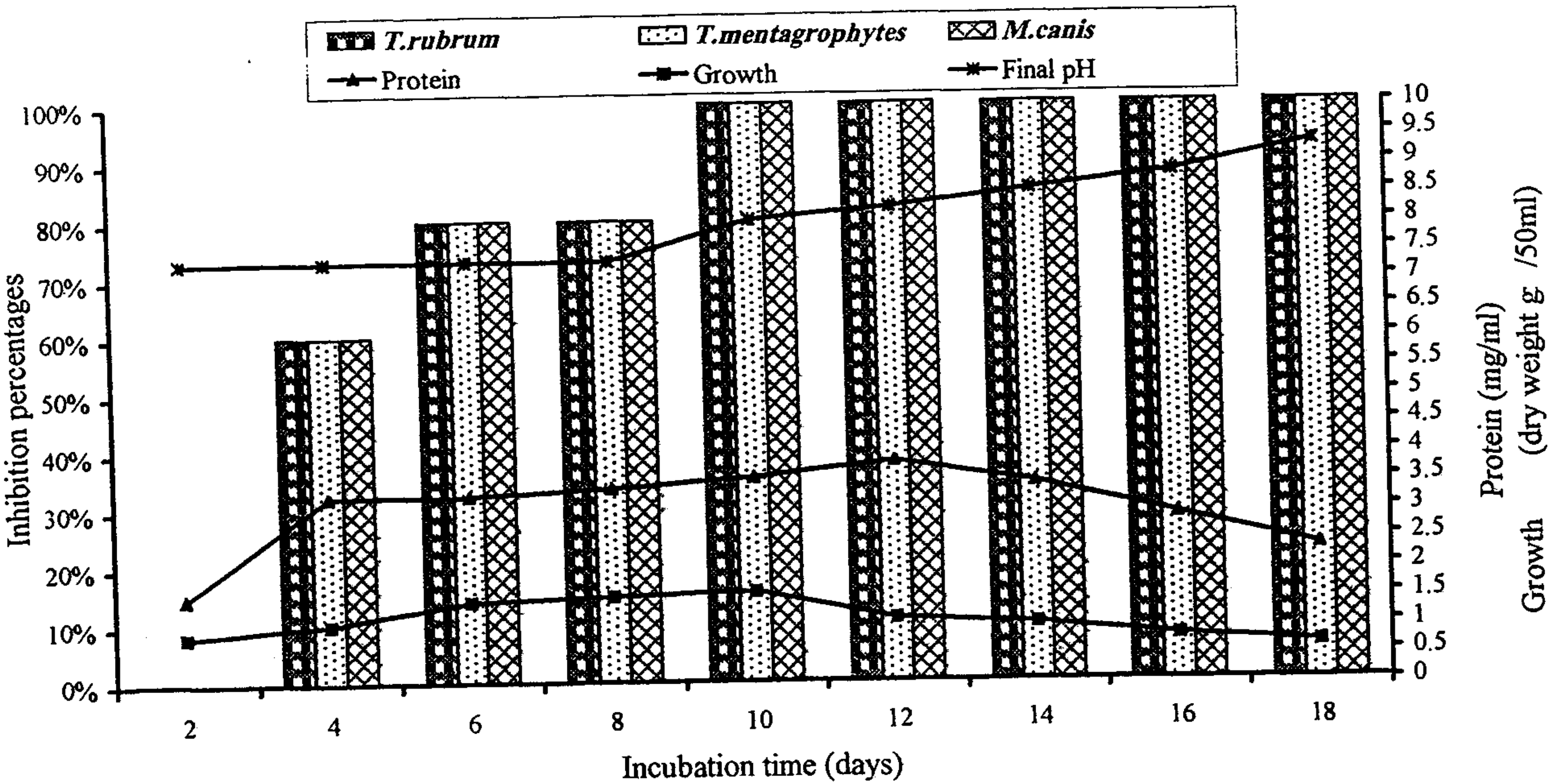


Fig. 7 . Effect of different incubation periods on antifungal activity, extracellular protein, growth rate and final pH of ***Streptomyces kanamyceticus*** EHE-68 in shaking condition at 180 rpm.

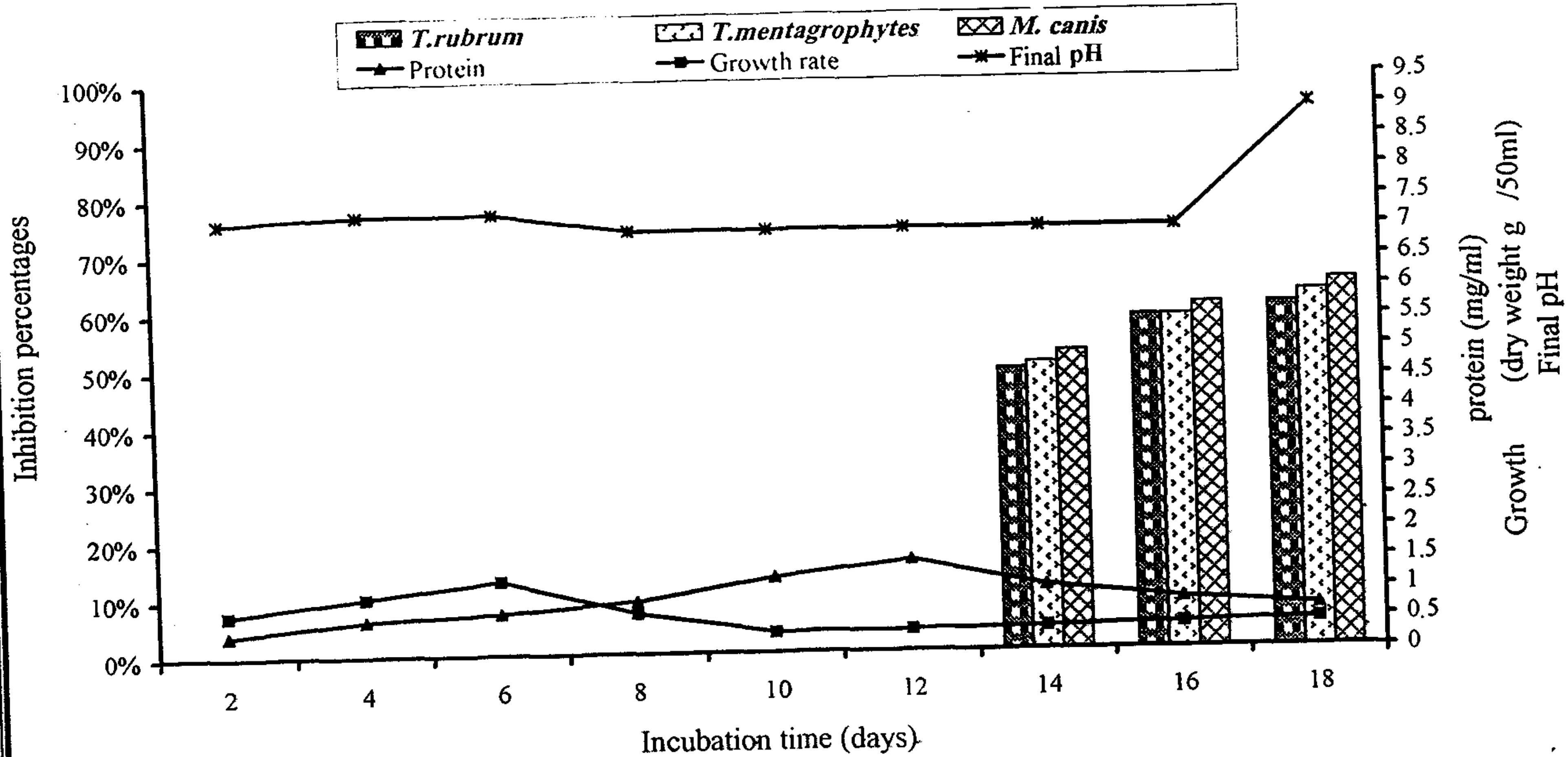


Fig. 8 . Effect of different incubaion periods on antifungal product, extracellular protein, growth rate and final pH of ***Streptomyces kanamyceticus*** EHE-68 in static condition

1.2 Effect of different incubation temperatures on the production of antifungal, extracellular protein, final pH and growth rate of *Sterptomyces kanamyceticus* EHE-68

Since, the optimum incubation period for antifungal production was 10 days, the next logic step was to investigate the effect of incubation temperature on the antifungal potentiality by the experimental organism. The starch nitrate medium was inoculated with *Streptomyces kanamyceticus* EHE-68 spore suspension, and incubated at 20, 25, 30, 35, 40, 45 and 50°C.

The culture flasks were incubated at 180 rpm. at a shaking incubator for 10 days. After which, antifungal production, extracellular protein, and growth rate were determined as shown in table (12) and figure (9). The results indicate that *Streptomyces kanamyceticus* EHE-68 was able to grow at 20, 25, 30, 35 and 40°C. The growth inhibited completely at 45 and 50°C. It is clear that 30°C was the optimum incubation temperature where the maximum value of antifungal activity, extracellular protein and growth rate were obtained. Then clear drop in antifungal activity, protein amount and growth rate was observed.

Table (12): Average values of antifungal biosynthesis, extracellular protein, final pH and growth of *Streptomyces kanamyceticus* EHE-68 incubated at different incubation temperatures

Temperature °C	Growth (dry weight) (g /50ml)	Final pH	Protein (mg/ml)	Growth inhibition rate (%) of		
				T.rubrum	*T.mentagrophytes*	*M.canis*
20°C	0.97	7.5	3.4495	92%	92%	92%
25°C	1.03	8.0	3.4814	95%	93%	95%
30°C	1.11	8.0	3.6566	100%	100%	100%
35°C	1.01	7.0	2.4067	90%	90%	92%
40°C	0.67	7.0	2.3177	0%	0%	0%
45°C	0.0	7.0	0.0036	0%	0%	0%
50°C	0.0	7.0	0.0025	0%	0%	0%

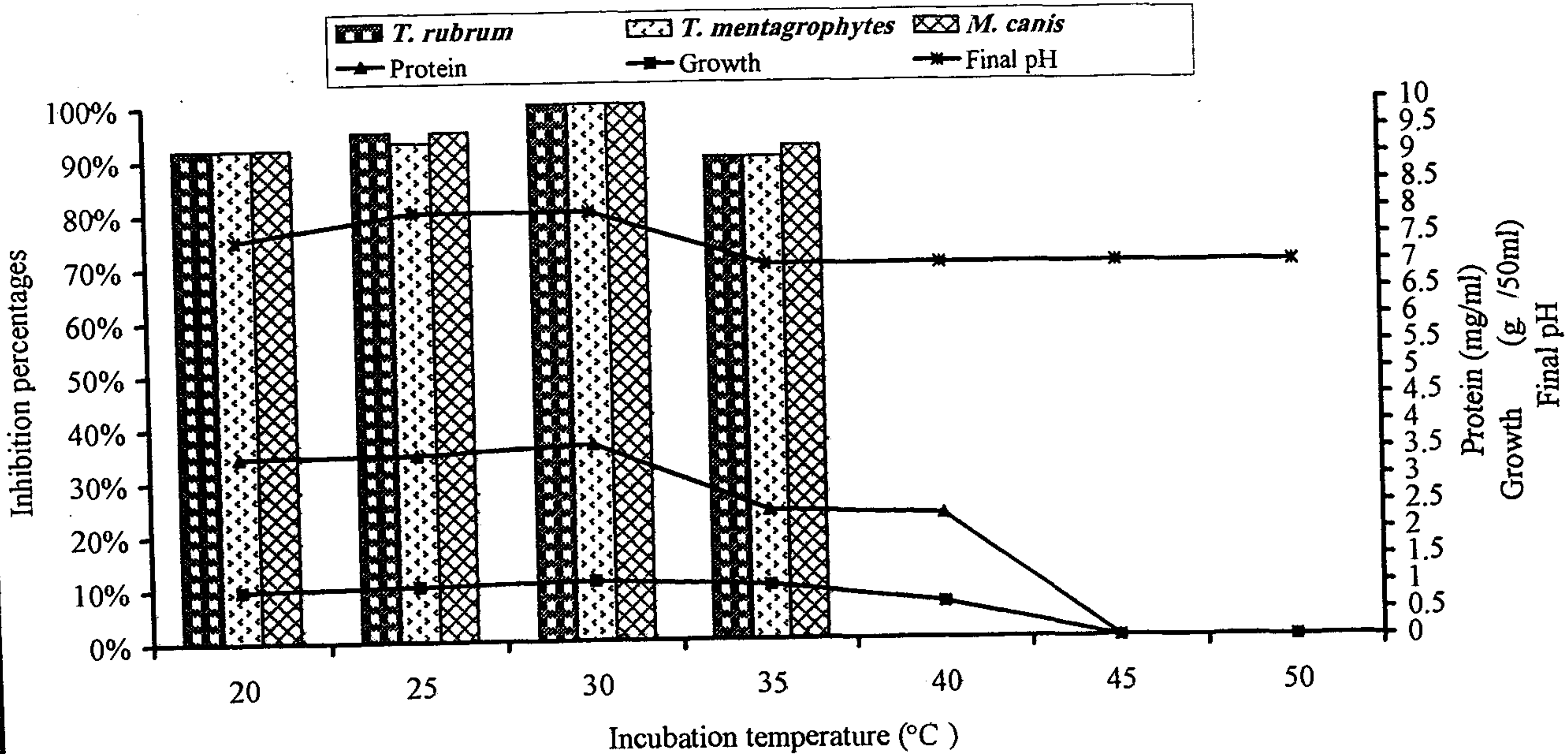


Fig. 9 . Effect of different incubation temperature on antifungal production, extracellular protein, growth rate and final pH of Streptomyces kanamyceticus EHE-68

1.3 Effect of initial pH values on the antifungal production, extracellular protein and final pH and growth rate of *Streptomyces kanamyceticus* EHE-68

This experiment was carried out to study the effect of initial pH values of the cultures medium on the antifungal production, the extracellular protein, final pH and growth rate of ***Streptomyces kanamyceticus*** EHE-68. The pH values were adjusted to cover the range from 4 to 10 pH before sterilization using 1.0 N HCl and 1.0 N NaOH. Triplicate flasks were used for particular pH value. The agitation speed, incubation period and temperature were those obtained from the previous experiments to be optimal for antifungal production by ***Streptomyces kanamyceticus*** EHE-68.

The results presented in table (13) and figure (10) indicate clearly that the optimum pH-value range from 7-9 for maximum production of antifungal compound, extracellular protein and growth of ***Streptomyces kanamyceticus*** EHE-68.

The optimal pH value at which the organism formed the highest value of antifungal compound was 7 and stable at 8 and 9 but decreased at 10. Maximum extracellular protein and growth rate was attained at pH 8, increasing or decreasing of pH values having deterioration effect on these parameters.

Table 13 . Average values of antifungal biosynthesis, extra-cellular protein, final pH and growth rate at different initial pH values of *Streptomyces kanamyceticus* EHE-68

pH	Growth (dry weight) (g /50ml)	Final pH	Protein (mg/ml)	Growth inhibition rate (%) of		
				T.rubrum	*T.mentagrophytes*	*M.canis*
4	0.68	6.5	1.0859	0%	0%	0%
5	0.72	7.0	2.8044	80%	80%	86%
6	0.98	7.5	2.5413	90%	88%	90%
7	0.98	8.0	3.2224	100%	100%	100%
8	1.00	8.0	3.3863	100%	100%	100%
9	0.97	8.0	2.9602	100%	100%	100%
10	0.71	8.5	2.5336	90%	90%	90%

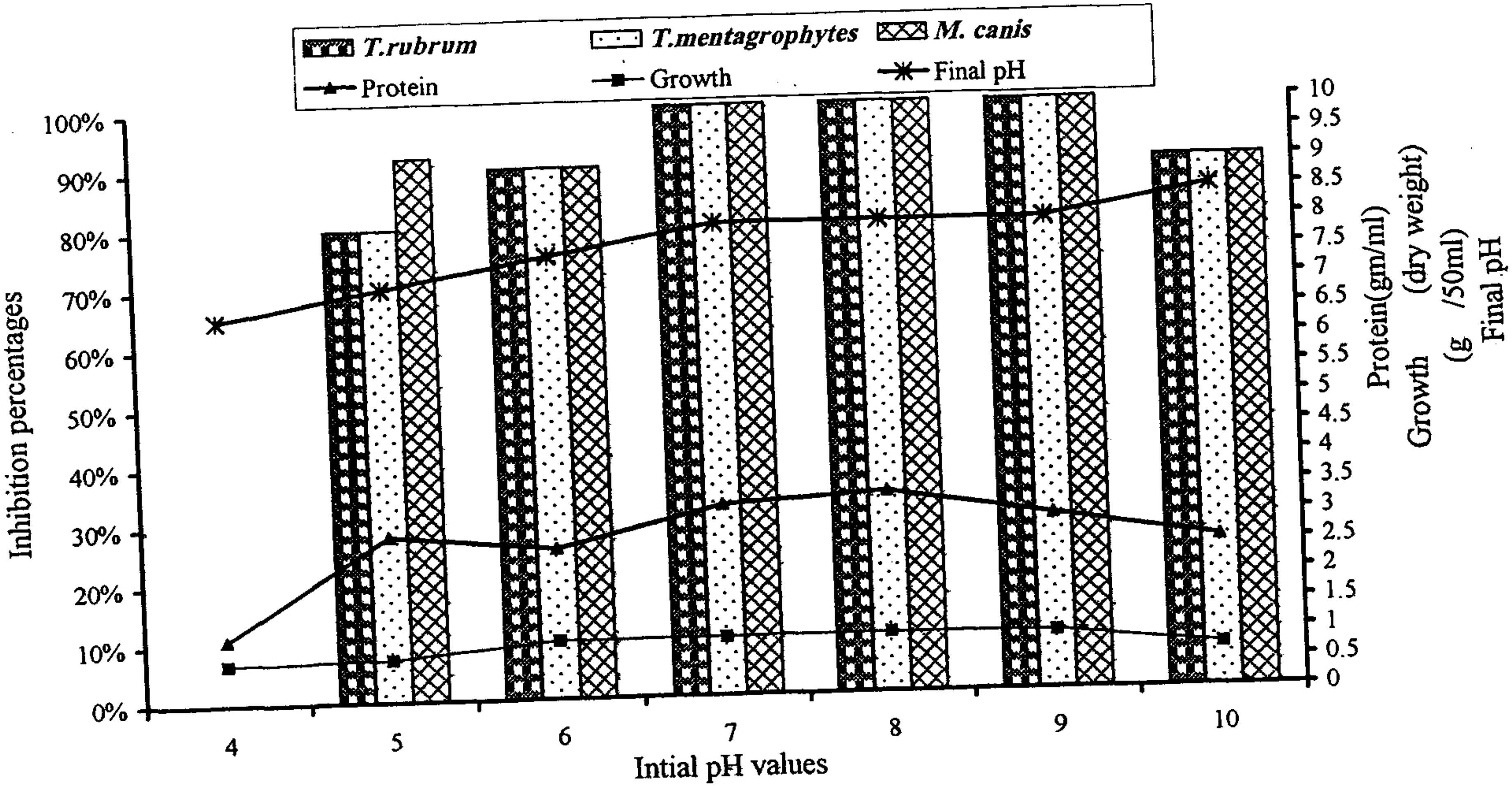


Fig. 10 . Effect of different hydrogen ion concentrations(pH) on antifungal product, extracellular protein, growth rate and final pH of ***Streptomyces kanamyceticus*** EHE-68

2. Nutritional Requirements

2.1 Effect of different carbon sources on antifungal production, extracellular protein, growth rate and final pH of *Streptomyces kanamyceticus* EHE-68.

In this experiment, the experimental organism (*S. kanamyceticus* EHE-68) was cultivated on starch-nitrate medium supplemented with different carbon soyrces. Control treatment was made without any carbon source. Each carbon source was added in a concentration of 2%. Triplicate flasks were made for each treatment.. the culture flasks were adjusted to pH 7.0 and incubated at 30?C at shaking incubator (180)rpm for 10 days.

The results are tabulated in table (14) and graphically in figure (11). The ability of the organism to grow and produce antifungal agent appeared to be carbon source specific. Among mannitol, lactose, galactose, starch, chitin, and maltose were found that starch, chitin and maltose the most suitable carbon source for producing antifungal agent by *S. kanamyceticus* EHE-68.

In presence of starch, the maximal values of the antifungal activity, extracellular protein and growth rate were obtained and the organism failed to produce antifungal compound at sucrose, cellulose and glucose when added to the medium as sole carbon sources.

The sources of carbon on which the organism exhibited the highest values of growth, antifngal production and extracellular protein can be arranged in the descending order starch > chitin > maltose > mannitol > galactose > lactose.

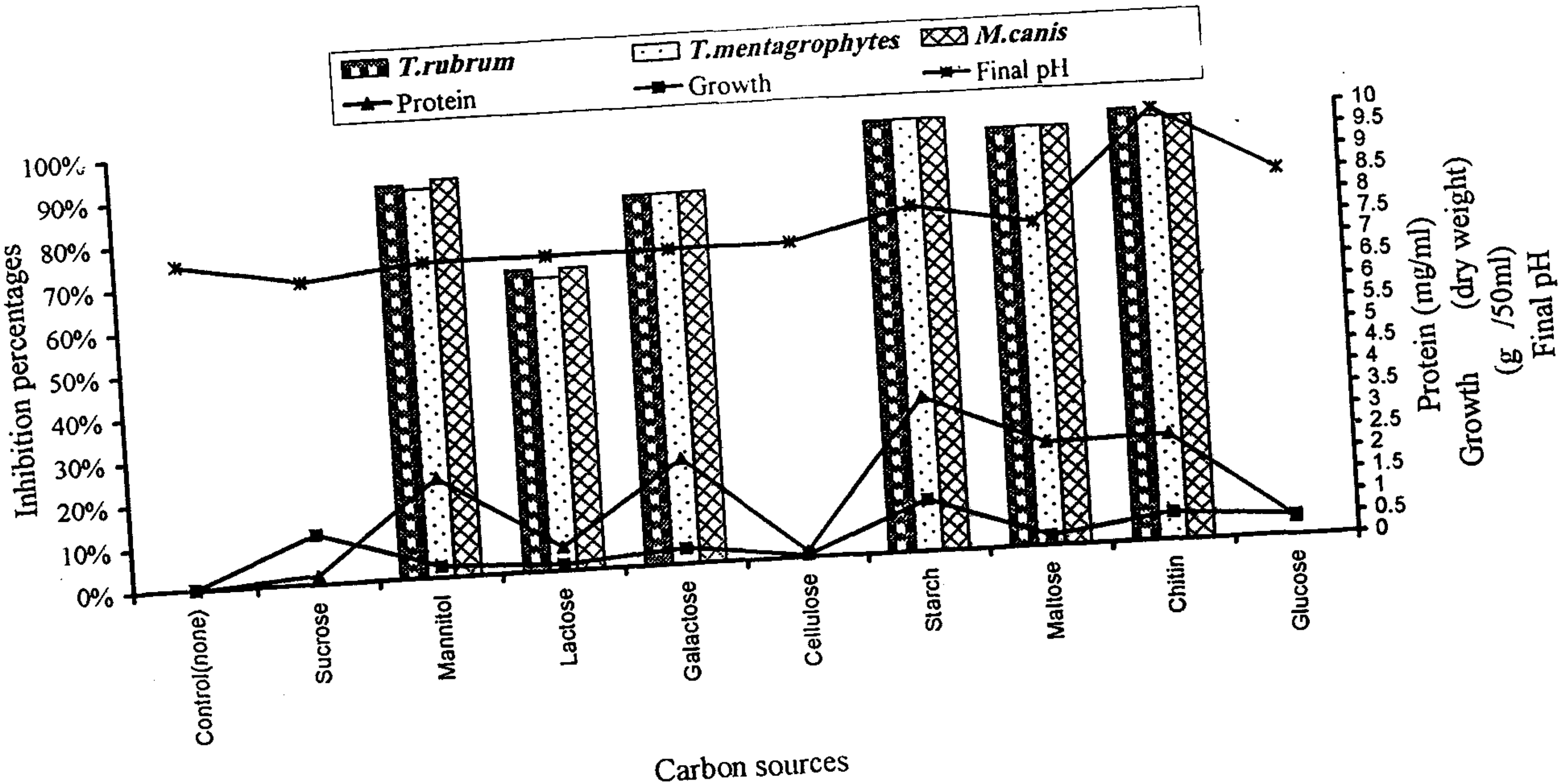


Fig. 11 . Average values of antifungal activity, extracellular protein, growth rate and final pH of ***Streptomyces kanamyceticus*** EHE-68 cultivated on different carbon sources.

2.2 Effect of different nitrogen sources on antifungal production, extracellular protein, growth rate and final pH of *Streptomyces kanamyceticus* EHE-68

This experiment was adopted in order to clarify the effect of different nitrogen sources on the growth, extracellular protein, final pH and antifungal compound produced by ***Streptomyces kanamyceticus*** EHE-68. The different nitrogen sources were supplemented separately to nitrogen-free starch-nitrate medium, in nitrogen content equivalent to 0.2 g % potassium nitrate to the modified basal medium.

The nitrogen sources used were organic as casein, peptone, beef-extract, yeast-extract, urea and asparagine and inorganic as ammonium nitrate, ammonium chloride, ammonium sulfate, ammonium citrate, sodium nitrate and potassium nitrate.

The obtained results, in table (15) and presented graphically in figure (12), illustrate that, ***Streptomyces kanamyceticus*** EHE-68 was unable to grow or to synthesize the antifungal compound on nitrogen free medium (control). Among the organic sources of nitrogen, casein gave the highest growth value, extracellular protein and antifungal biosynthesis by the experimental organism.

Inorganic sources of nitrogen as ammonium nitrate and potassium nitrate initiate the experimental organism to produced maximal values of antifungal activity aganist ***T. rubrum, T. mentargophytes*** and ***M. canis***. On the other hand ammonium and potassium nitrate are the best nitrogen sources for production of extracellular protein among organic and inorganic tested nitrogen sources.

Table (15): Average values of antifungal biosnthesis, extracellular protein, final pH and growth rate of *Streptomyces kanamyceticus* EHE-68 cultivated on different nitrogen sources

Nitrogen sources	Growth (dry weight) (g /50ml)	Final pH	Protein (mg/ml)	Growth inhibition rate (%) of		
				T. rubrum	*T. mentagrophytes*	*M. canis*
Casein	1.15	7.3	2.2306	100%	100%	100%
Peptone	1.06	7.5	0.9291	97%	97%	98%
Beef-extract	0.78	7.5	1.2895	93%	94%	95%
Urea	0.90	6.5	0.8394	88%	89%	91%
Yeast-extract	0.73	9.0	1.9861	91%	91%	93%
Asparagine	0.95	7.0	1.5333	92%	93%	97%
Amm. nitrate	0.71	7.5	2.9253	100%	100%	100%
Amm. chloride	0.91	7.5	1.2876	98%	98%	99%
Amm. sulfate	0.22	8.5	1.9405	100%	100%	100%
Amm. citrate	0.41	9.5	3.1769	97%	100%	100%
Sodium nitrate	0.34	7.0	2.6131	96%	95%	96%
Potassium nitrate	1.02	8.0	3.5566	100%	100%	100%
Control (none)	0.01	7.5	0.0000	0%	0%	0%

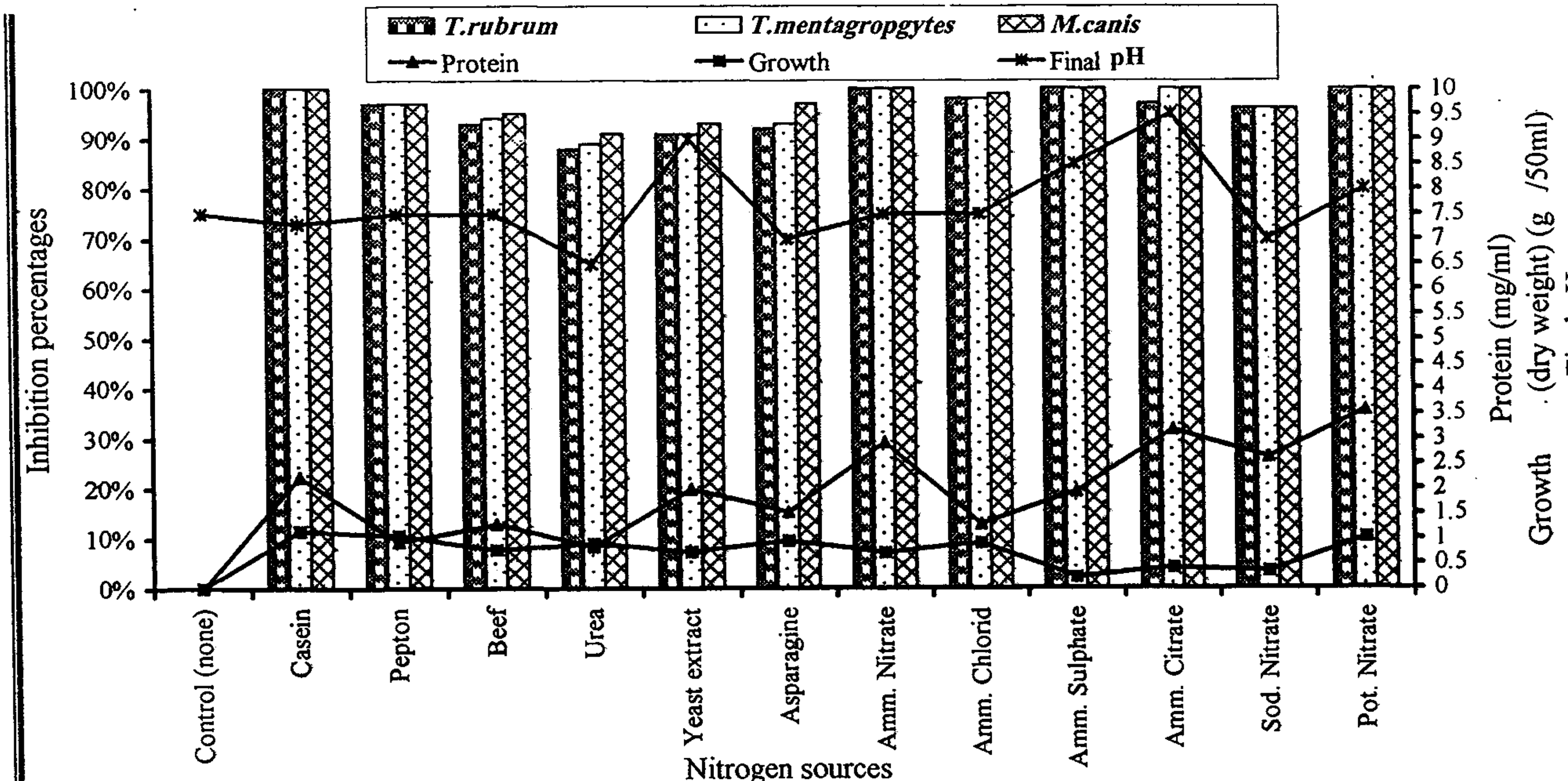


Fig. 12 . Average values of antimicrobial potentiality, extracellular protein, growth rate and final pH of ***Streptomyces kanamyceticus*** EHE-68 cultivated on different nitrogen sources.

Amino acids as nitrogen sources

This experiment was adopted in order to clarify the effect of different amino acids as nitrogen sources on the growth, extracellular protein, final pH and antifungal compound produced by ***Streptomyces kanamyceticus*** EHE-68. The different nitrogen sources were supplemented separately to nitrogen free medium, in nitrogen content equivalent to 0.2 % potassium nitrate to the modified basal medium.

Amino acids used were L- cystein, L- valine, L-phenylalanine, L- methionine, L- histidine, L- arginine, L-aniline, L-glycine and L- glutamic acid.

The obtained results areults are in table (16) and graphically in figure (13) illustrating that, the ***Streptomyces kanamyceticus*** EHE-68 was unable to grow or to synthesis the antifungal compound on nitrogen free medium (control). Among the amino acids tested, L-glutamic acid gave the highest growth value (1.06 g /50ml), extracellular protein (3.6110 mg/ml) and antifungal biosynthesis compound against all tested dermatophyte fungi by the experimental organism. On the other hand, L-phenylalanine, L- methionine and L- histidine were completely inhibited biosynthesis of antidermatophyte compound produced by ***St. kanamyceticus*** EHE-68.

Table 16 . Average values of antifungal biosynthesis, extracellular protein, final pH and growth rate of *Streptomyces kanamyceticus* EHE-68 cultivated on different amino acids as nitrogen sources

Amino acides	Growth (dry weight) (g / 50ml)	Final pH	Protein (mg / ml)	Growth inhibition rate (%) of		
				T. rubum	*T. mentagrophytes*	*M. canis*
L-Cystein	0.33	6.8	3.4457	88%	86%	89%
L-Valine	0.41	6.5	3.0510	100%	100%	100%
L-Phenylalanine	0.98	7.0	3.4457	0%	0%	0%
L-Methionine	0.66	6.5	3.3564	0%	0%	0%
L-Histidin	0.45	7.0	2.7508	0%	0%	0%
L-Arginine	0.98	6.7	3.4456	70%	72%	74%
L-Aniline	0.21	7.5	2.5533	100%	100%	100%
L-glycine	0.41	7.5	2.6660	100%	100%	100%
L-glutamic acid	1.06	7.0	3.6110	100%	100%	100%
Control (none)	0.01	7.5	0.0000	0%	0%	0%

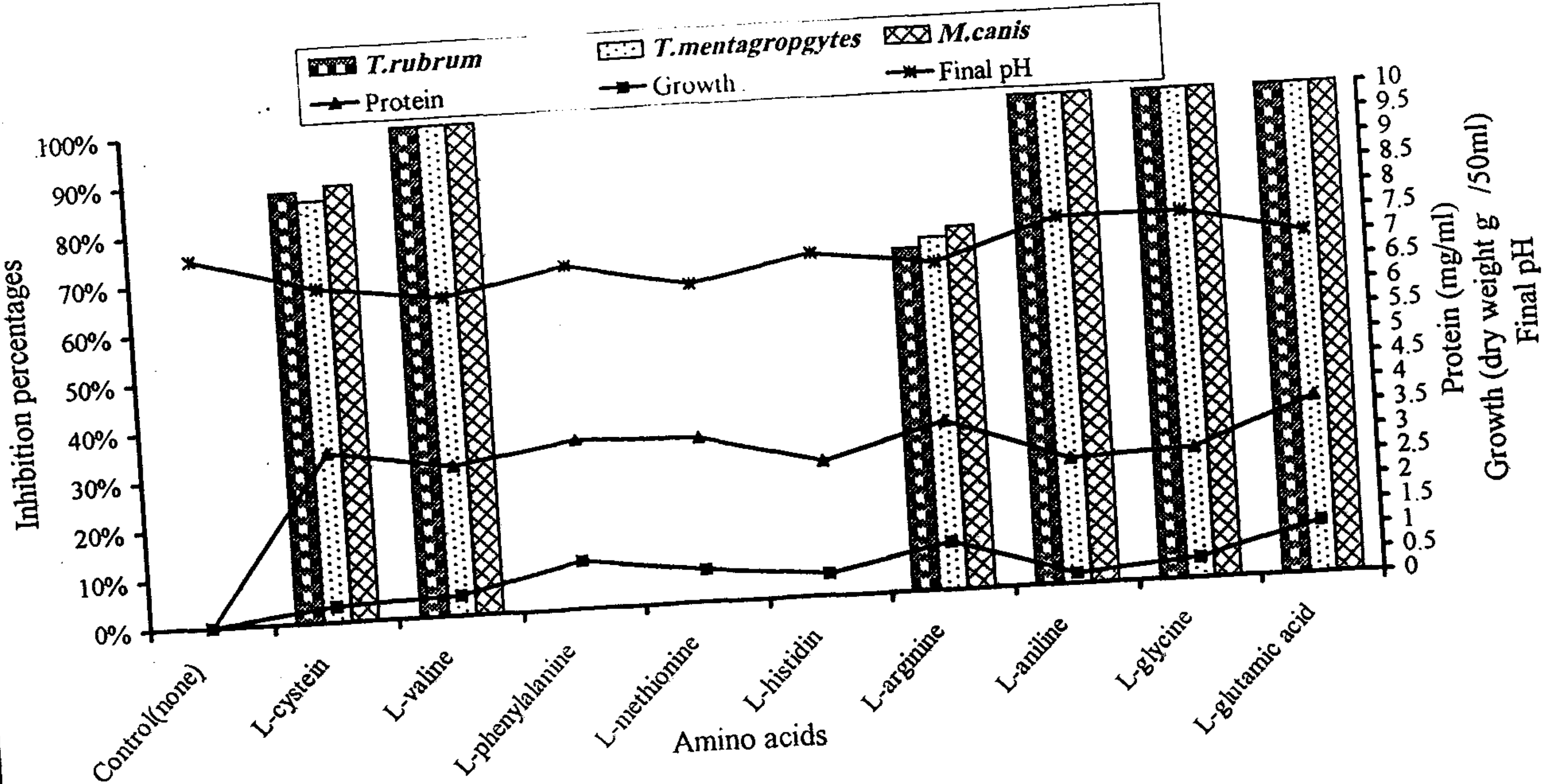


Fig. 13 . Average values of antifungal activity, extracellular protein, growth rate and final pH of ***Streptomyces kanamyceticus*** EHE-68 cultivated on different amino acids as nitrogen sources

2.3 Effect of different phosphorus sources on antifungal production, extracellular protein, growth rate and final pH of *Streptomyces kanamyceticus* EHE-68

In this experiment a basal nutrient medium was prepared free of phosphorous source and supplemented by equimolecular levels of phosphorous sources to test its influence on antifungal compound production, extracellular protein, growth rate by ***Streptomyces kanamyceticus*** EHE-68.

The used phosphorous sources were as follows: potassium monobasic phosphate, potassium dibasic phosphate, sodium monobasic phosphate, sodium dibasic phosphate and ammonium phosphate.

The obtained results recorded in table (17) and graphically in figure (15) showed that, the ***Streptomyces kanamyceticus*** EHE-68 was not grow well in the absence of phosphorous sources (control), synthesis of antifungal decreased also. The most favorable phosphorous source for antifungal biosynthesis, extracellular protein and growth rate was K_2HPO_4. Generally, the different phosphorous sources can be arranged according to antifungal activity as follow: K_2HPO_4 > KH_2PO_4 = NaH_2PO_4 > $NH_4H_2PO_4$ > Na_2HPO_4, but in the order of extracellular protein production was as follows: K_2HPO_4 > NaH_2PO_4 > $NH_4H_2PO_4$ > Na_2HPO_4 > KH_2PO_4.

Table 17 . Average values of antifungal biosynthesis, extracellular protein, final pH and growth rate of *Streptomyces kanamyceticus* EHE-68 cultivated on different phosphorous sources

Phosphorous sources	Growth (dry weight) (g /50ml)	Final pH	Protein (mg/ml)	Growth inhibition rate (%) of		
				T.rubrum	*T.mentagrophytes*	*M.canis*
K_2HPO_4	1.67	8.5	3.3248	100%	100%	100%
KH_2PO_4	1.32	8.5	1.5213	100%	100%	100%
$NH_4H_2PO_4$	1.23	6.5	2.0748	92%	92%	95%
Na_2HPO_4	1.64	9	1.8696	90%	90%	93%
NaH_2PO_4	1.29	8.5	2.1673	100%	100%	100%
Control	0.78	7.5	1.0146	90%	88%	92%

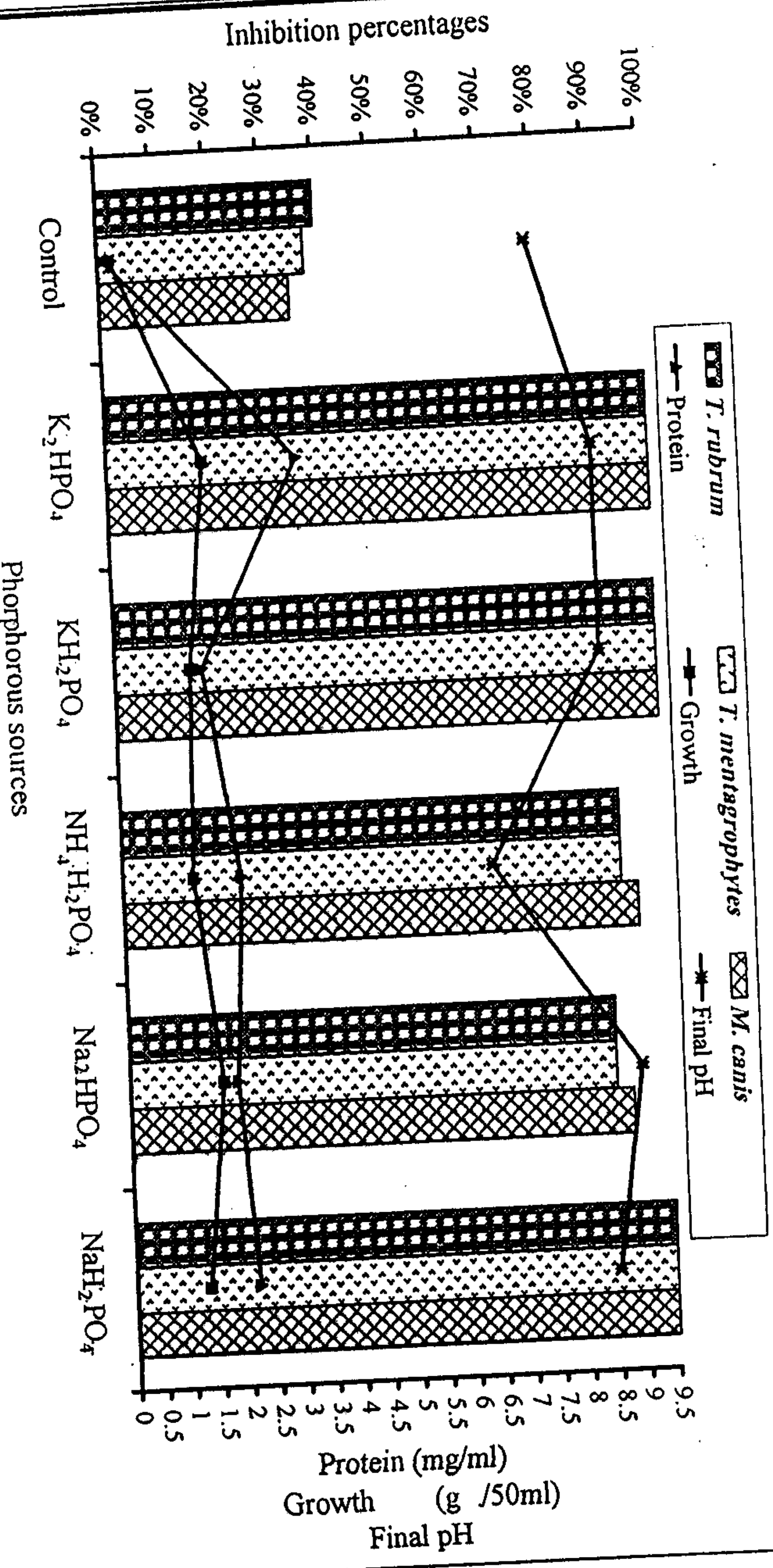


Fig. 14 . Average values of antifungal activity, extracellular protein, growth rate and final pH of ***Streptomyces kananyceticus*** EHE-68 cultivated on different phosphorous sources

2.4 Effect of different metallic-ions on anti-microbial potentiality, extracellular protein, growth rate and final pH of *Streptomyces kanamyceticus* EHE-68

It is well known that metallic–ions play an important role in growth and rate of germination of microbial spores, as well as shortening the sporophore formation time. The chemical elements function as essential nutrients for cell synthesis, and as regulatory mechanisms for various transformations that take place in the living system. Different metallic-ions were added to the starch nitrate medium in (1mg/100ml). These elements were nickel chloride, copper sulphate, cobalt chloride, sodium arsenate, zinc sulphate, ferrous sulphate, lead acetate and calcium chloride. Triplicate flasks were made for each particular treatment. Control treatment was maintained in parallel. General culture conditions were carried out as usual. At the end of the incubation period, the cultures were filtered and centrifuged. The culture filtrates were used for estimation of antifungal activity and protein amounts. The results recorded in table (18) and figure (16) indicate that nickel chloride, sodium arsenate induced maximum value of antifungal potentiality of the experimental organism, but the all remaining metallic-ions induced inhibitory effects on antifungal agent. On the other hand, the maximum amount of protein was obtained in the presence of sodium arsenate.

Table 18. **Average values of antifungal biosynthesis, extracellular protein, final pH growth rate of *Streptomyces kanamyceticus* EHE-68 cultivated on different metallic-ions**

Metallic-ions	Growth (dry weight) (g/50ml)	Final pH	Protein (mg/ml)	Growth inhibition (%) of		
				T.rubrum	*T.mentagrophyes*	*M.canis*
Nickel chloride	1.38	6.5	1.2394	100%	100%	100%
Copper sulphate	1.00	6.5	0.1172	79%	79%	80%
Cobalt sulphate	0.79	6.5	1.6739	100%	100%	100%
Sodium arsenate	1.33	6.5	2.3338	100%	100%	100%
Zinc sulphate	0.00	6.0	0.2293	0%	0%	0%
Ferrous sulphate	0.71	7.5	0.5883	67%	66%	70%
Lead acetate	0.00	6.5	0.1754	0%	0%	0%
Calcium sulphate	0.73	7.0	0.6257	72%	70%	70%
Control (none)	0.88	8.0	1.0792	96%	95%	98%

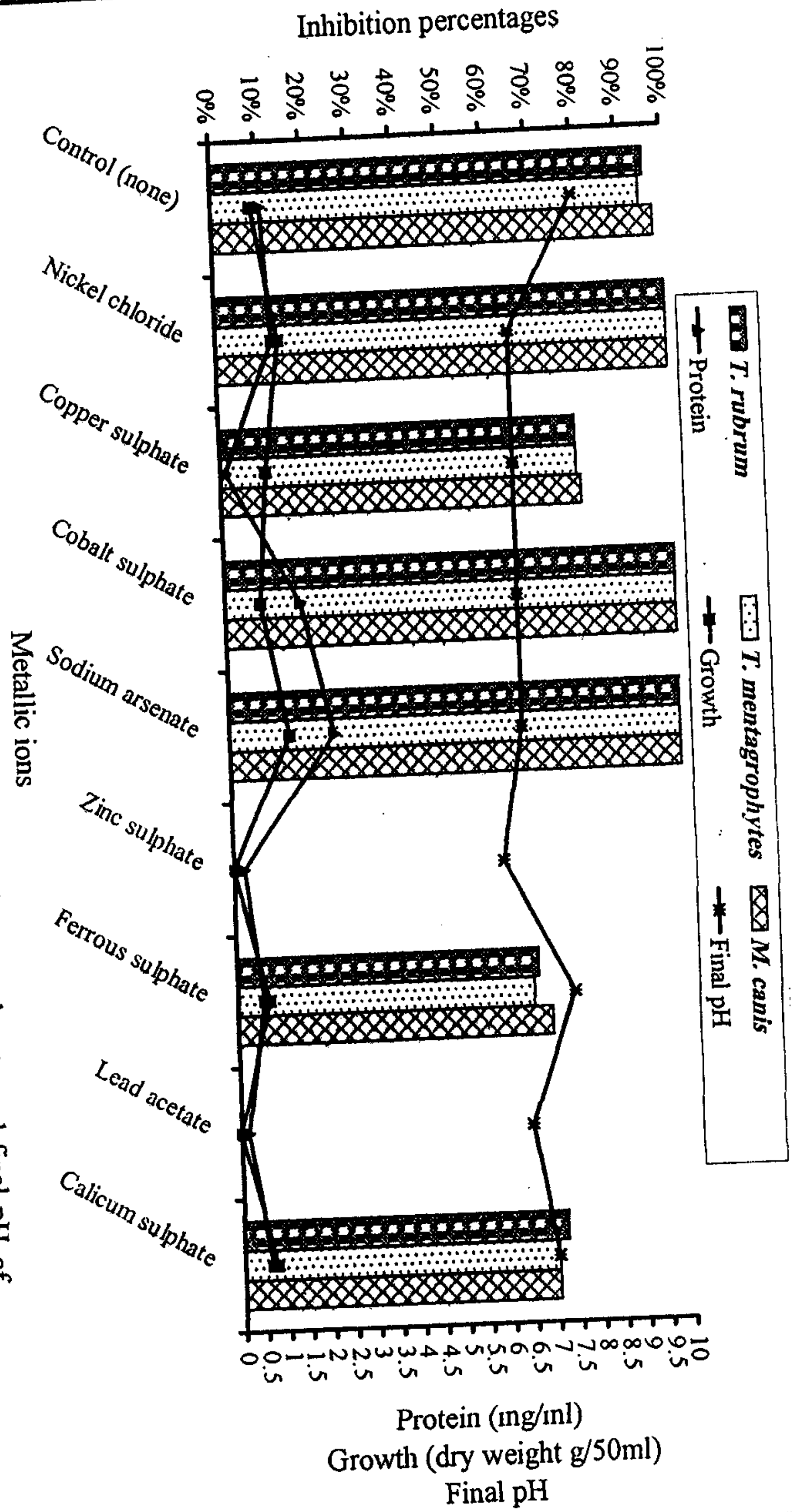


Fig. 15. Average values of antifungal production, extracellular protein, growth rate and final pH of ***Streptomyces kanamyceticus*** EHE-68 cultivated on different sources of metalic ions

Table 19 . Effect of different concentrations of essential oils on the growth of tested dermatophyte fungi

Essential oils with different concentrations (µl/100ml media)	Percentages of growth inhibition of		
	T.rubrum	*T.mentagrophytes*	*M. canis*
Marjoram oil			
31.25	42%	47%	31%
62.50	64%	75%	42%
125.00	88%	77%	46%
250.00	93%	100%	54%
Lemon oil			
31.25	0%	0%	0%
62.50	22%	425	69%
125.00	55%	70%	77%
250.00	67%	74%	79%
Orange oil			
31.25	0%	0%	0%
62.50	33%	22%	50%
125.00	57%	40%	62%
250.00	72%	47%	70%
Mint oil			
31.25	79%	2%	100%
62.50	88%	18%	100%
125.00	100%	37%	100%

250.00	100%	51%	100%
Chamomile oil			
31.25	45%	21%	24%
62.50	51%	33%	33%
125.00	77%	49%	67%
250.00	100%	59%	67%
Flowers oil			
31.25	0%	40%	25%
62.50	47%	43%	61%
125.00	64%	45%	72%
250.00	88%	61%	72%
Clove oil			
31.25	100%	100%	100%
62.50	100%	100%	100%
125.00	100%	100%	100%
250.00	100%	100%	100%
Camphor oil			
31.25	5%	49%	61%
62.50	72%	67%	77%
125.00	95%	74%	100%
250.00	100%	95%	100%

Garlic oil			
31.25	24%	31%	37%
62.50	59%	35%	49%
125.00	81%	61%	64%
250.00	100%	66%	64%
Peach oil			
31.25	25%	35%	25%
62.50	55%	88%	31%
125.00	89%	97%	64%
250.00	96%	100%	64%

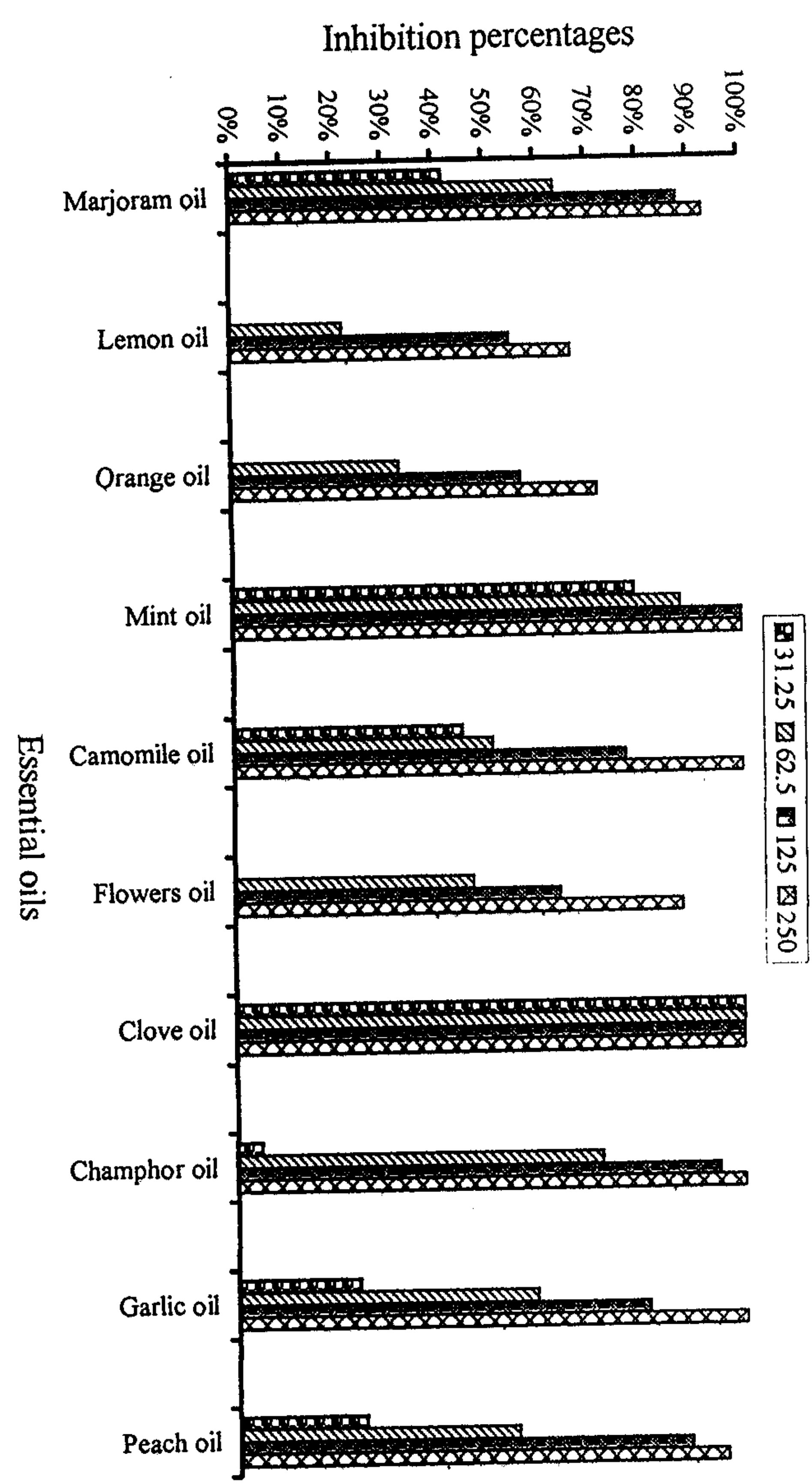


Fig. 16 .Antifungal effect of different concentration (µl/100ml medium)of different essential oils against ***T. rubrum***

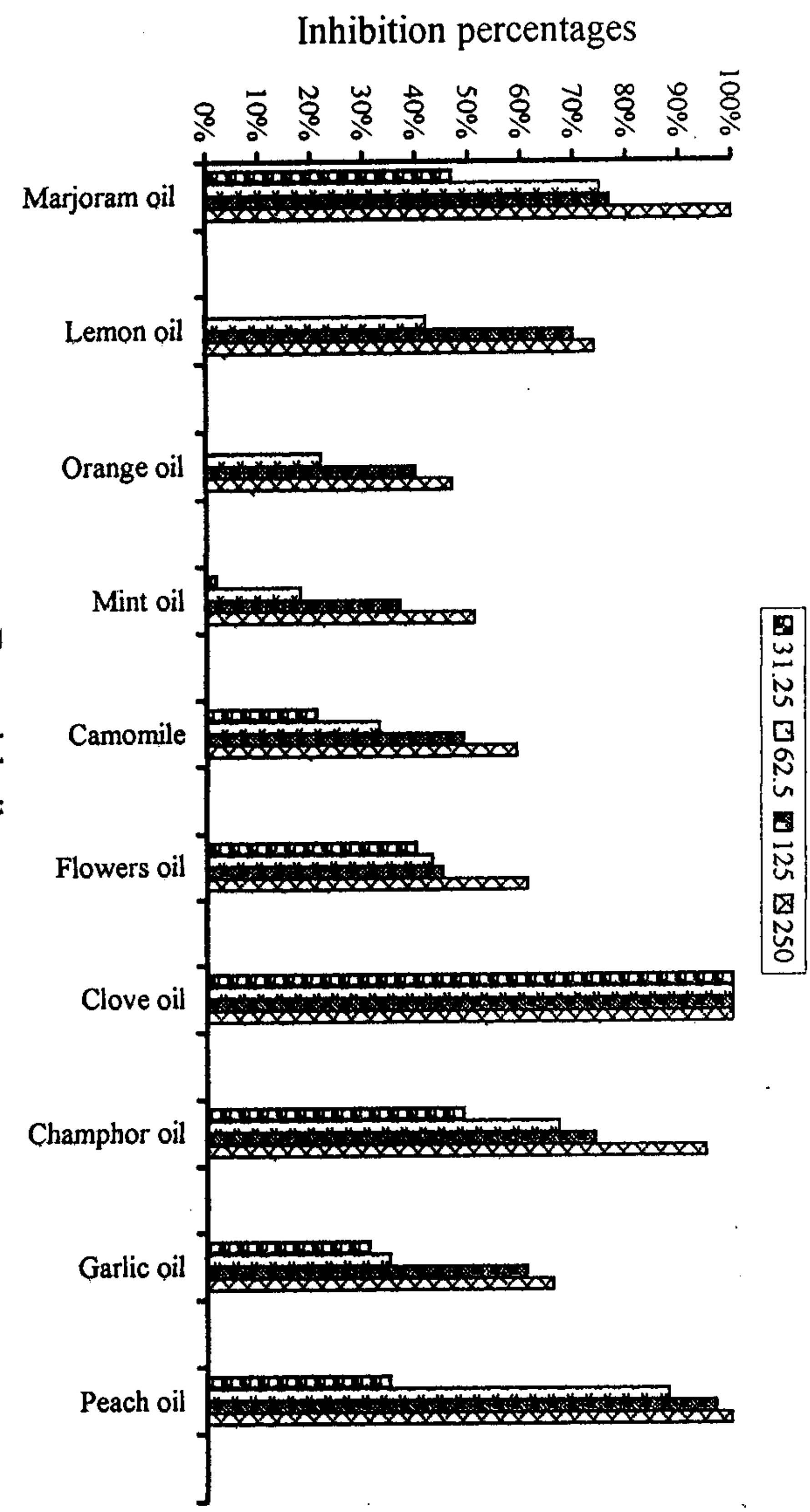


Fig. 17 .Antifungal effect of different concentrations (µl/100ml medium) of different essstial oils against ***T. metagrophytes***

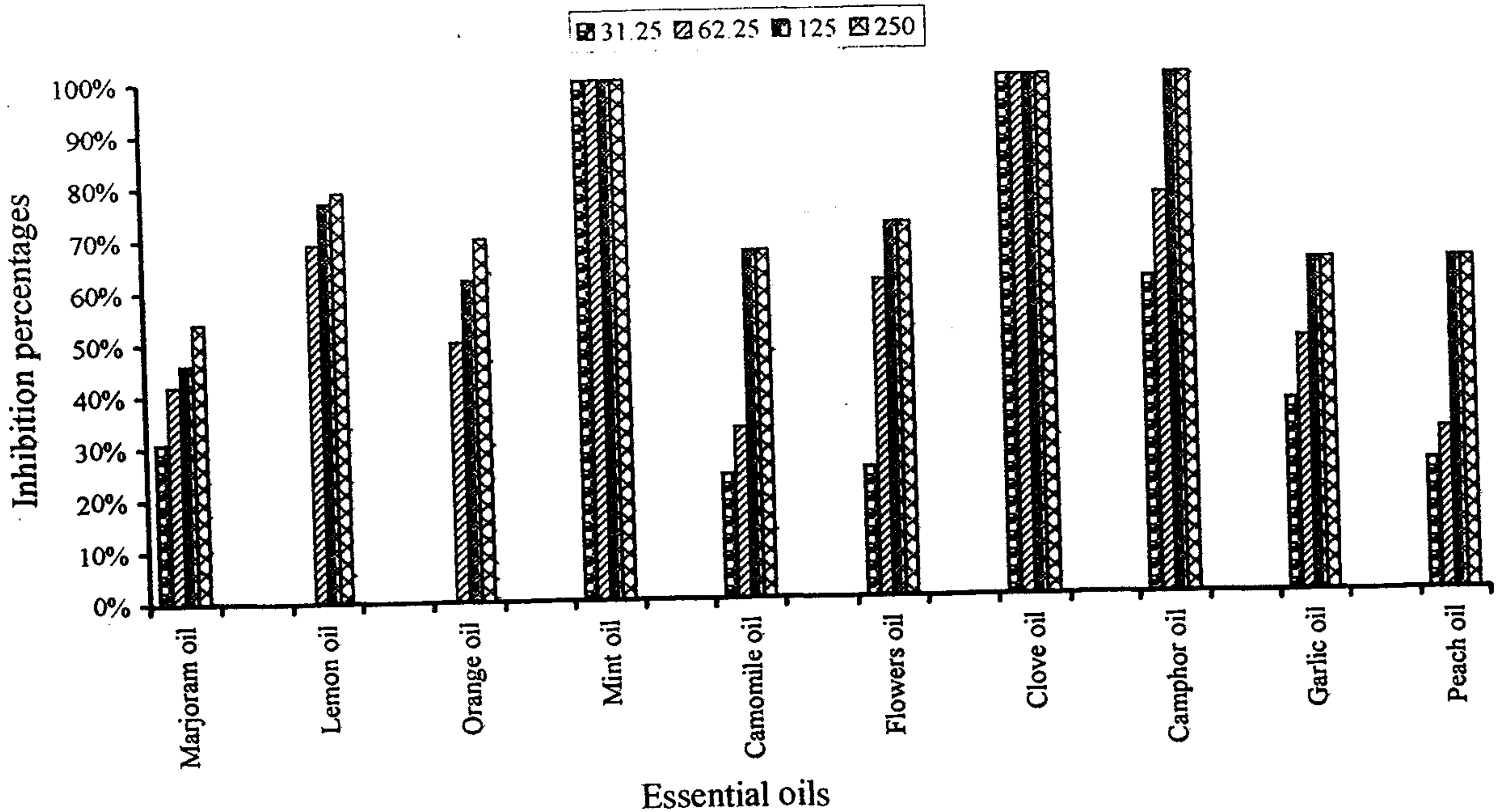
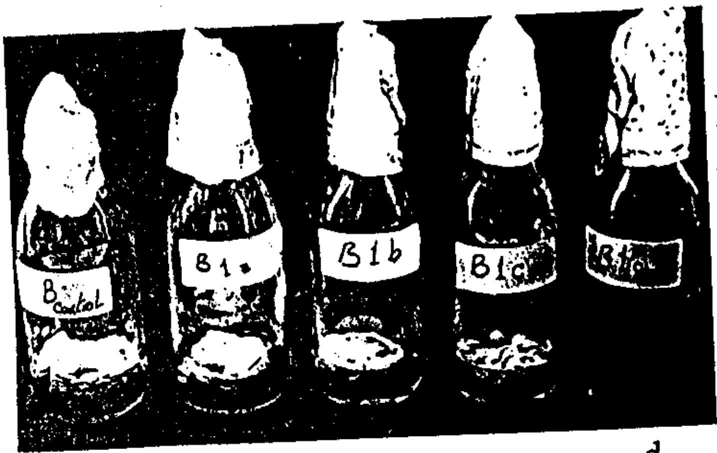


Fig. 18 . Antifungal effect of different concentrations (μl/100ml medium) of different essential oils on ***M. canis***

Control a b c d

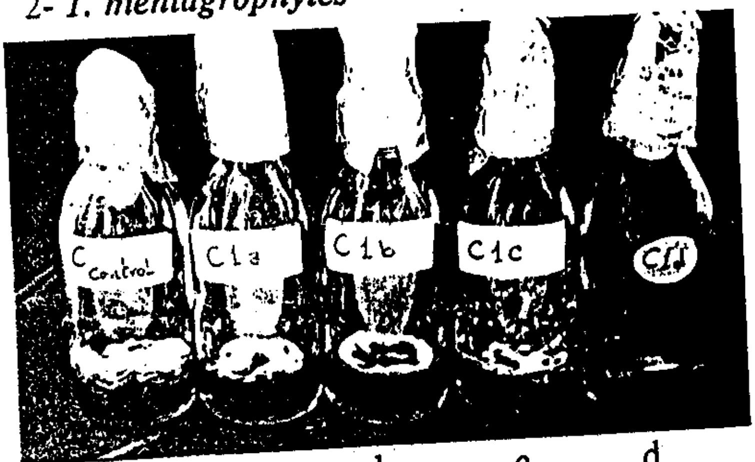
1- *T. rubrum*



Control a b c d

2- *T. mentagrophytes*

Fig.(15):
Effect of different concentrations of essential oils on growth of tested dermatophytes (from A-J).



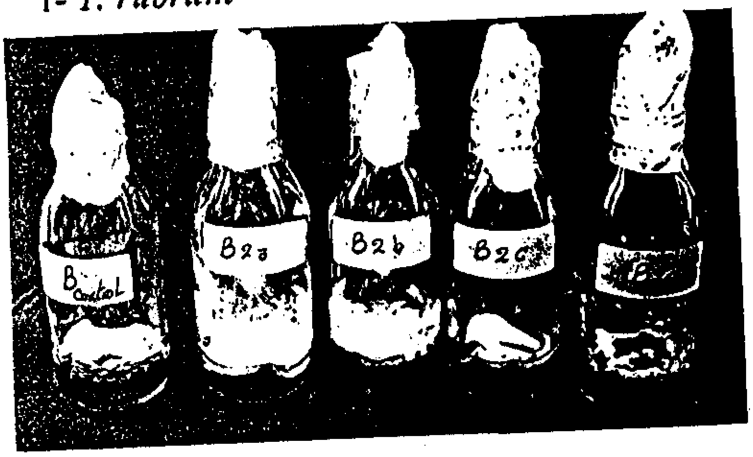
Control a b c d

3- *M.canls*

A: Inhibition of growth of (1,2 &3) at different concentrations of marjoram oil a (31.25), b (62.5), c (125) & d (250 μl/100ml).

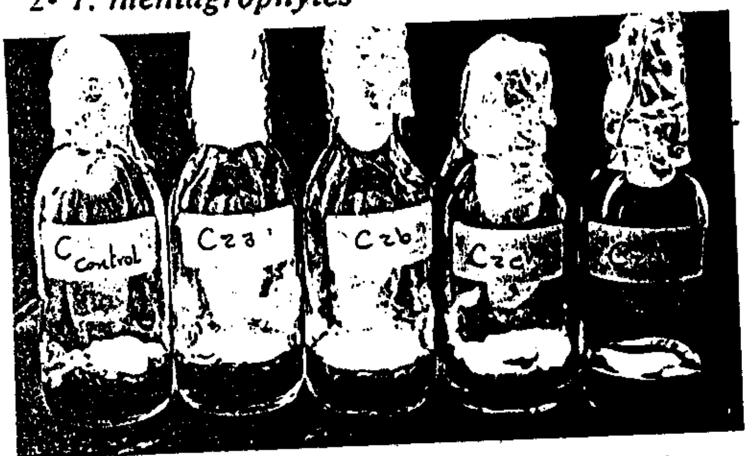
Control a b c d

1- *T. rubrum*



Control a b c d

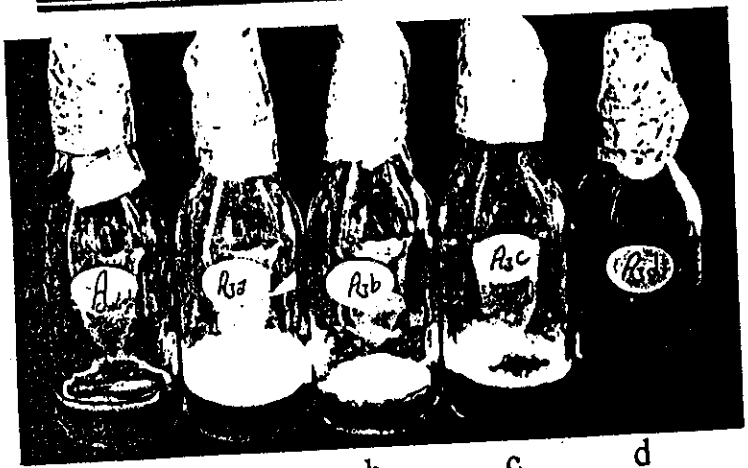
2- *T. mentagrophytes*



Control a b c d

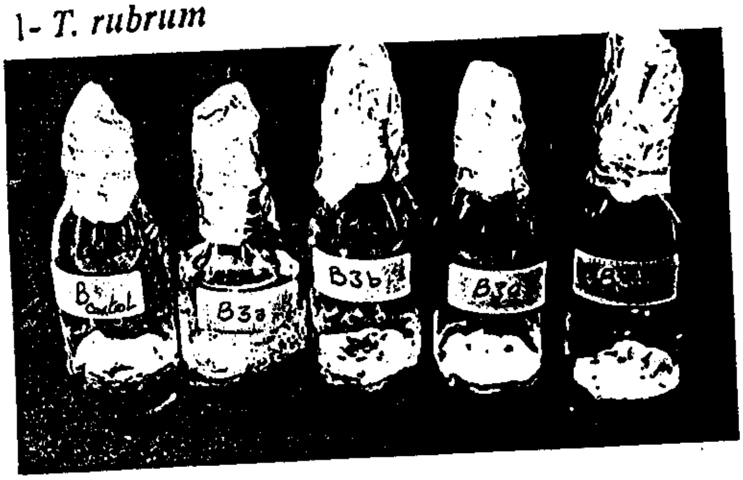
3- *M.canis*

B: Inhibition of growth of (1,2 &3) at different concentrations of Lemon oil a (31.25), b (62.5), c (125) & d (250 μl/100ml).



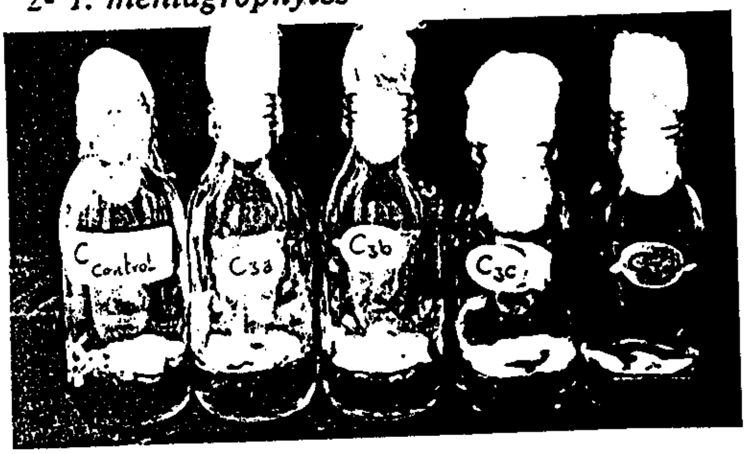
Control a b c d

1- *T. rubrum*



Control a b c d

2- *T. mentagrophytes*



Control a b c d

3- *M.canis*

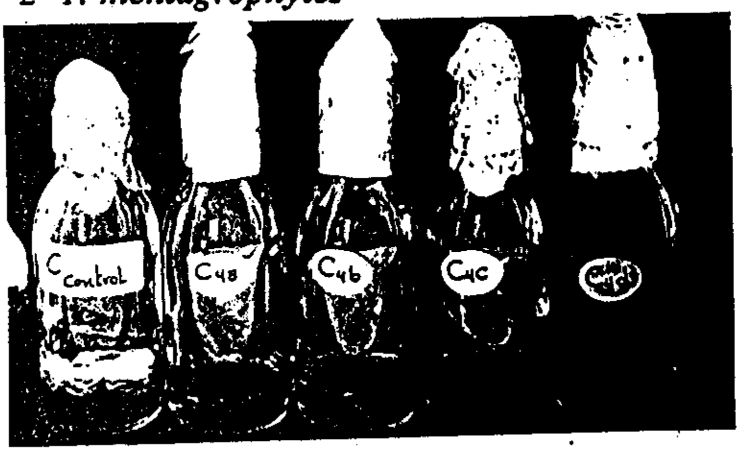
C: Inhibition of growth of (1,2 &3) at different concentrations of Orange oil a (31.25), b (62.5), c (125) & d (250 μl/100ml).

Control a b c d

1- *T. rubrum*

Control a b c d

2- *T. mentagrophytes*



Control a b c d

3- *M.canis*

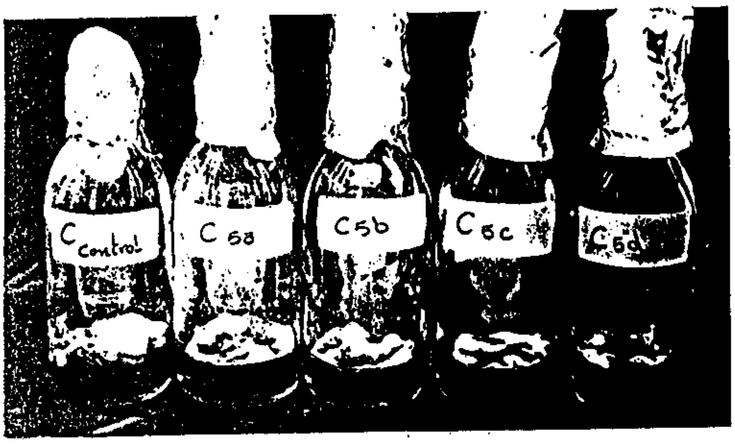
D: Inhibition of growth of (1,2 &3) at different concentrations of Mint oil a (31.25), b (62.5), c (125) & d (250 μl/100ml).

Control a b c d

1- *T. rubrum*

Control a b c d

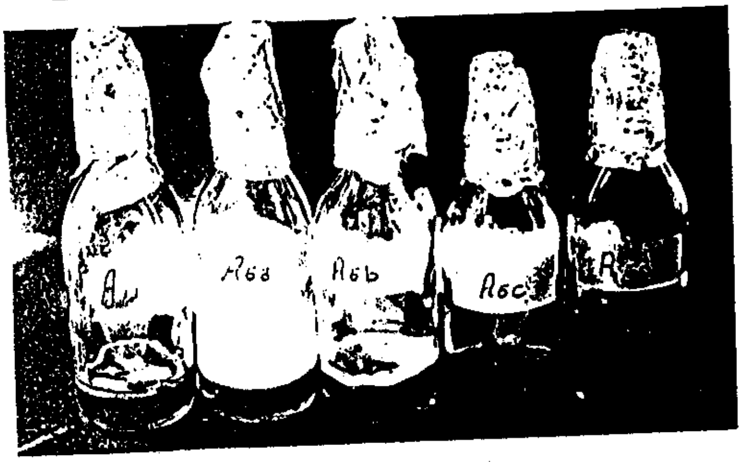
2- *T. mentagrophytes*



Control a b c d

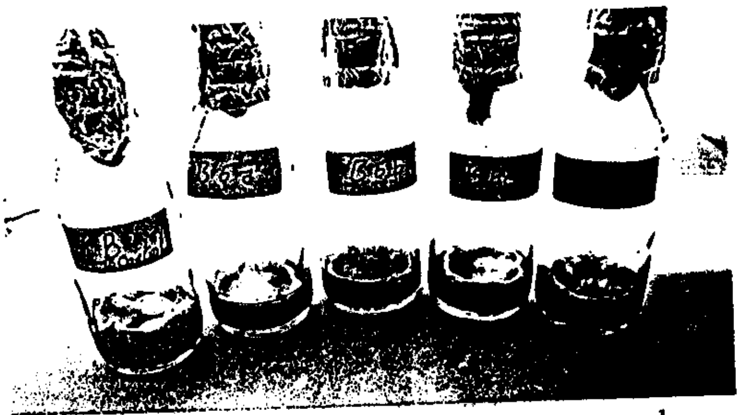
3- *M.canis*

E: Inhibition of growth of (1,2 &3) at different concentrations of Camomile oil a (31.25), b (62.5), c (125) & d (250 µl/100ml).



Control a b c d

1- *T. rubrum*



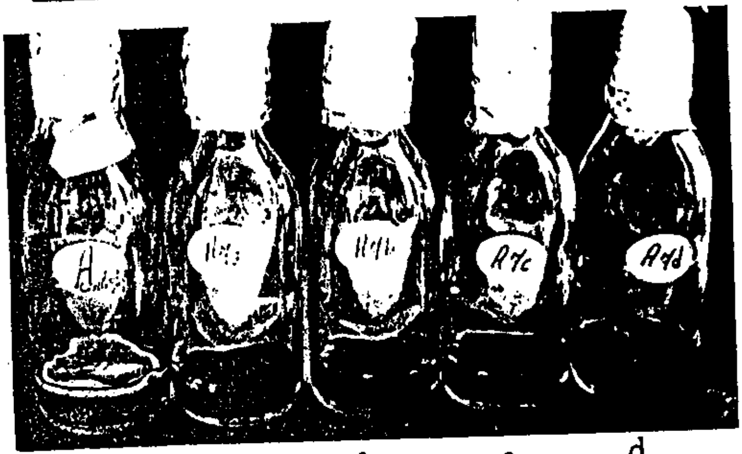
Control a b c d

2- *T. mentagrophytes*

Control a b c d

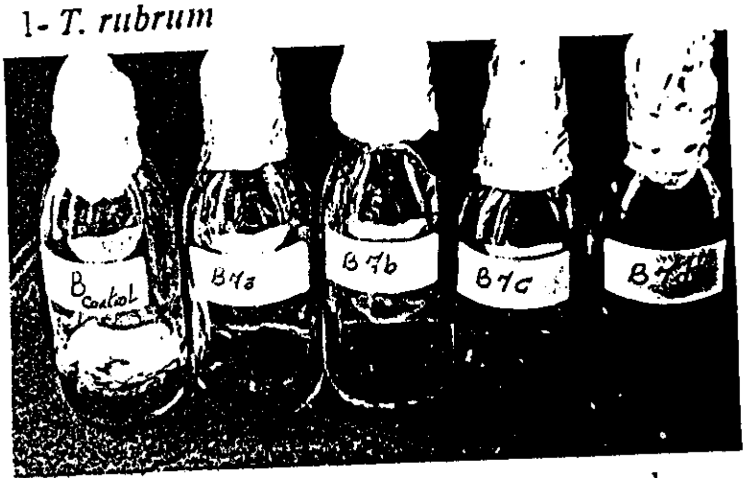
3- *M.canis*

F: Inhibition of growth of (1,2 &3) at different concentrations of Flowers oil a (31.25), b (62.5), c (125) & d (250 µl/100ml).



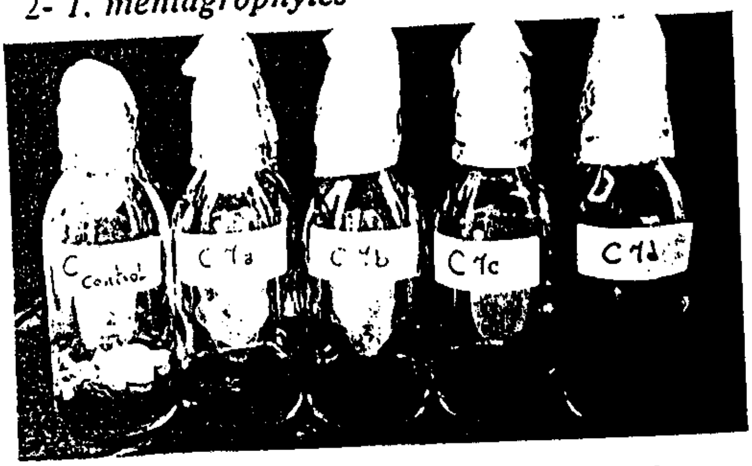
Control a b c d

1- *T. rubrum*



Control a b c d

2- *T. mentagrophytes*



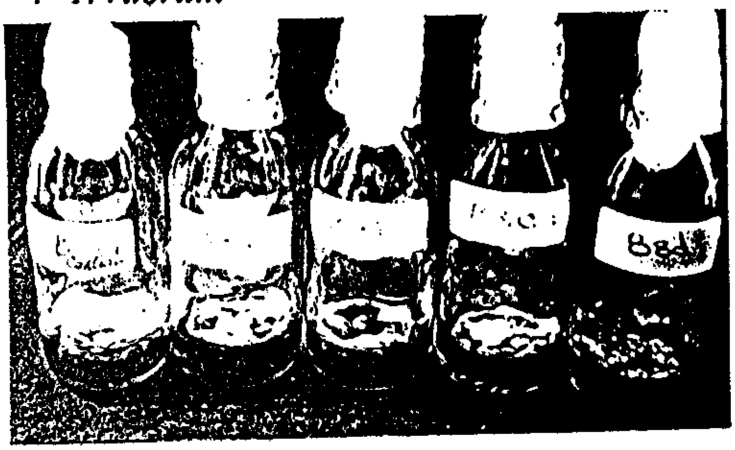
Control a b c d

3- *M.canis*

G: Inhibition of growth of (1,2 &3) at different concentrations of Clove oil a (31.25), b (62.5), c (125) & d (250 μl/100ml).

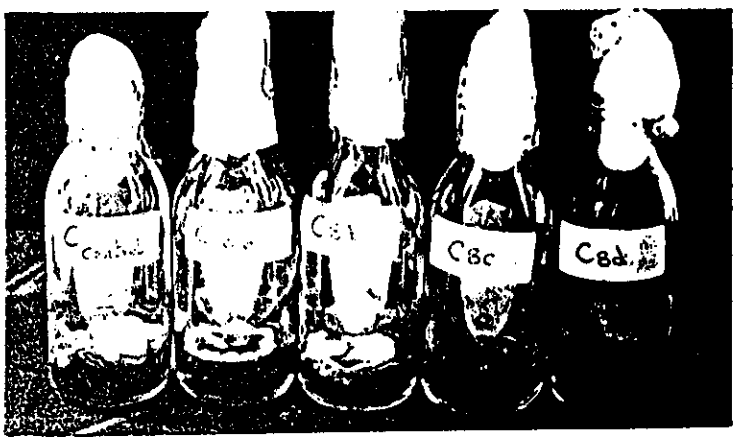
Control a b c d

1- *T. rubrum*



Control a b c d

2- *T. mentagrophytes*



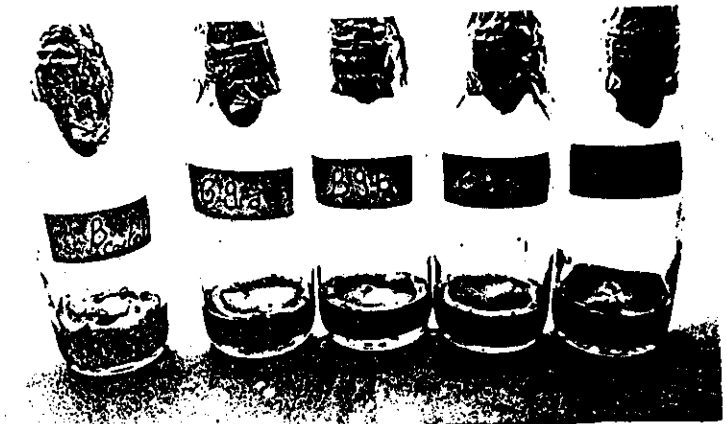
Control a b c d

3- *M.canis*

H: Inhibition of growth of (1,2 &3) at different concentrations of Camphor oil a (31.25), b (62.5), c (125) & d (250 µl/100ml).

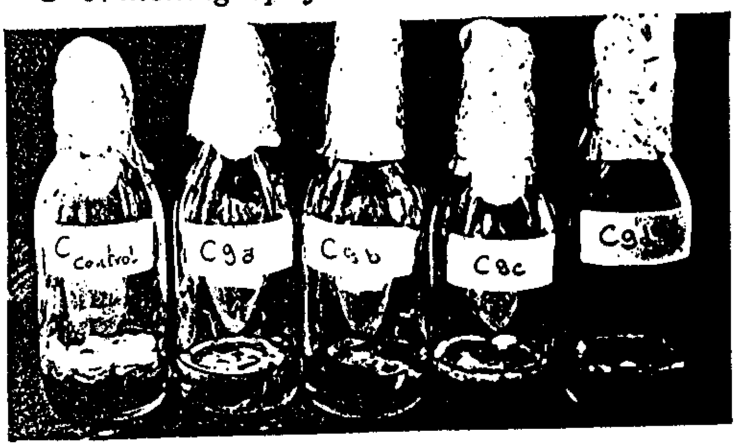
Control a b c d

1- *T. rubrum*



Control a b c d

2- *T. mentagrophytes*



Control a b c d

3- *M.cani*

I: Inhibition of growth of (1,2 &3) at different concentrations of Garlic oil a (31.25), b (62.5), c (125) & d (250 μl/100ml).

2. Effect of different concentrations of propolis on the growth rate of tested dermatophtye fungi

Table (20) and figure (20 & 21) indicates that propolis (PEE) have an inhibitory effect on the growth rate of ***M. canis*** at the following concentrations (50, 100, 200, 300, 400, 800 mg/100ml) by (4%, 29%, 35%, 49%, 56%, and 100%, respectively).

Propolis at concentrations (50, 100, and 200 mg/100ml) had no effect on *T.* ***mentagrophytes***, but at concentrations (300 & 400 mg/100ml) it had inhibition percent (37% & 64%, respectively). Finally, the complete inhibition was obtained at concentration (800 mg/100ml) which gave inhibition percent 100%.

The growth of ***T.rubrum*** affected by propolis at concentrations (50, 100, 200, 300, 400, 800 mg/100ml) by inhibition percentages (20%, 48%, 53%, 62%, 78%, and 100%, respectively).

Table 20 . Effect of different concentration of propolis (bee glue) on the growth of tested dermatophytes fungi

Concentration of propolis (g/100ml medium)	Inhibition percentages		
	T. rubrum	*T. mentagrophytes*	*M. canis*
50	20%	0%	4%
100	48%	0%	29%
200	53%	0%	35%
300	62%	37%	49%
400	78%	64%	56%
800	100%	100%	100%

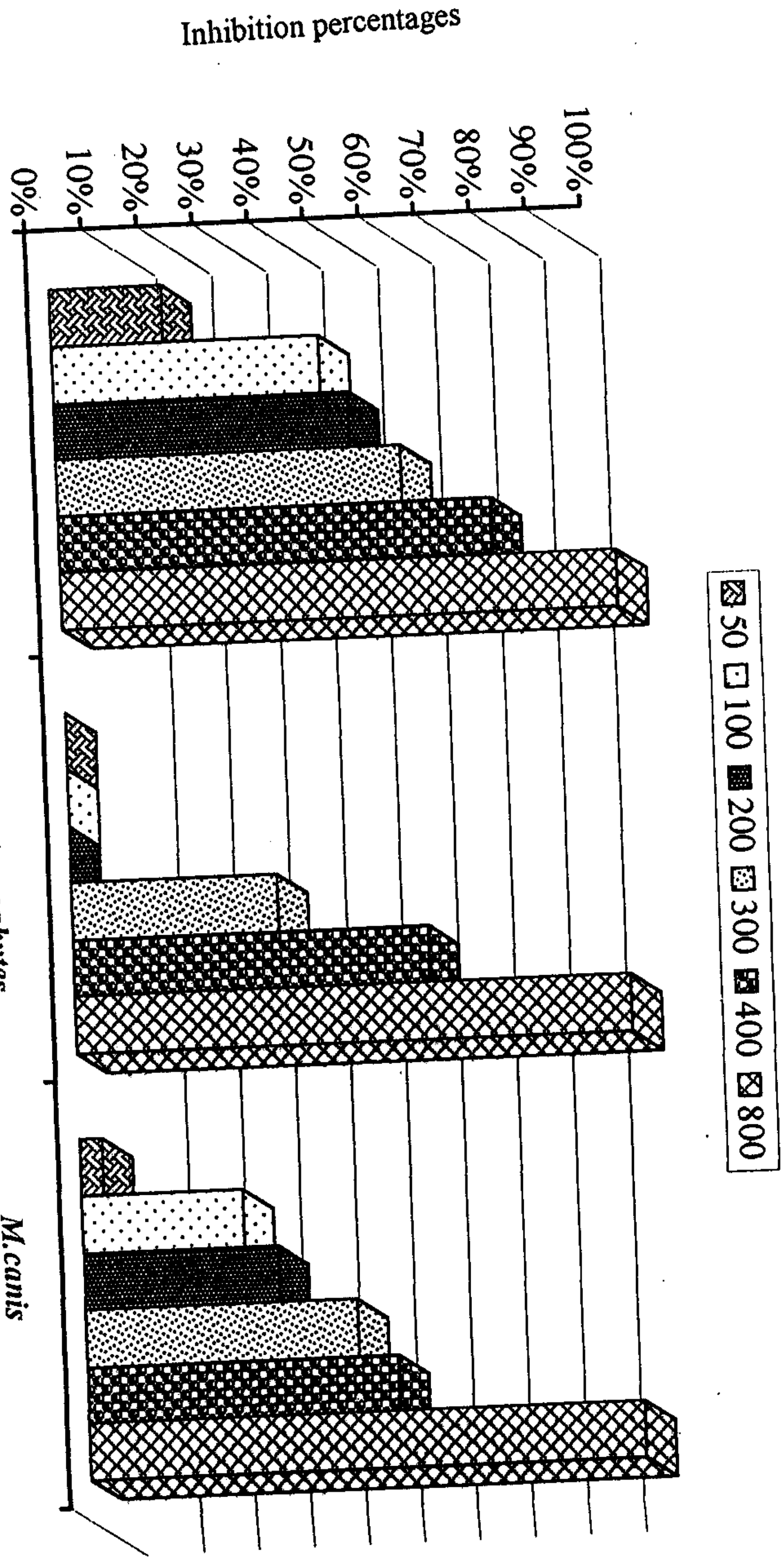
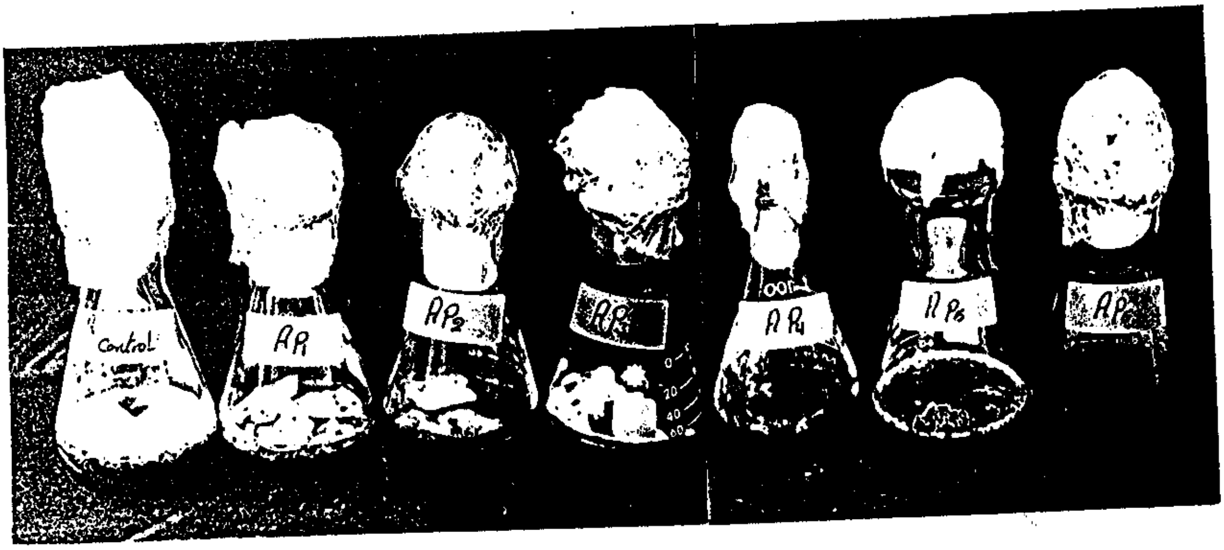
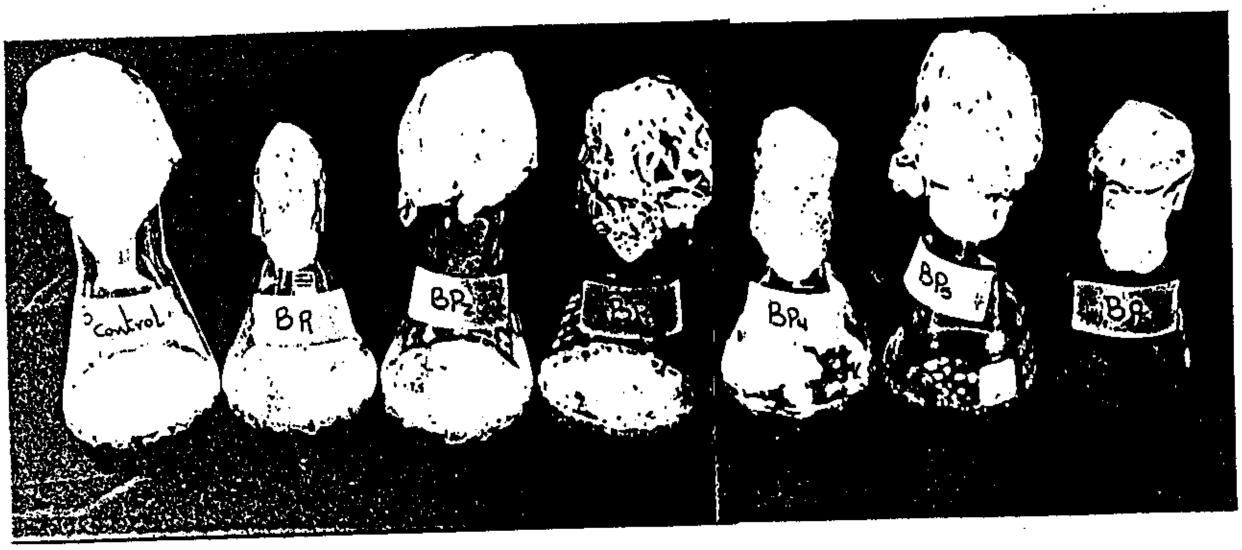


Fig. 20 . Antifungal effect of different concentrations of propolis ethanolic extract (propolis mg/100ml medium)



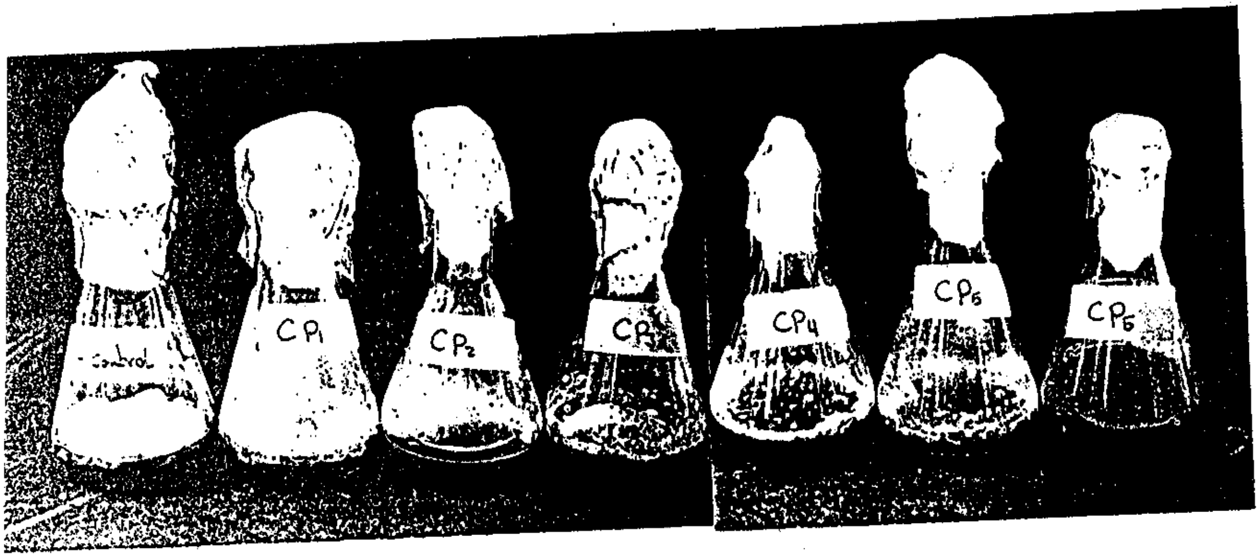
Control a b c d e f

1- *T. rubrum*



Control a b c d e f

2- *T. mentagrophytes*



Control a b c d e f

3- *M. canis*

Fig(19): Effect of different concentrations of propolis (a,b,c,d,e &f) on the growth rate of (1, 2 &3).

(a) = 50mg/100ml medium

(b) = 100mg/100ml medium

(c) = 200mg/100ml medium

(d) =300mg/100ml medium

(e) =400mg/100ml medium

(f) =800mg/100ml medium

3. Fungistatic and fungicidal activity of tested essential oils and propolis against *T. rubrum, T. mentagrophytes*, and *M. canis.*

The results of table (21) and figure (22) illustrated the fungicidal and fungistatic nature of the tested essential oils and propolis. Generally, almost of tested essential oils exhibited notable fungicidal and fungistatic activity against the tested fungi.

No mycelial dry weight was determined or detected as a result of exposure to clove oil at (250 μl/100ml) which means that it has a highly fungicidal effect on all tested dermatophyte fungi. Mint oil and champhor oil had fungicidal effect on ***T. rubrum*** and ***M. canis***, but it had no effect on ***T. mentagrophytes***. While garlic oil showed high fungicidal effect on ***T. rubrum*** but not on T. ***mentagrophytes*** and ***M. canis***, as well as peach oil had the fungicidal effect on ***T. mentagrophytes*** and fungistatic effect on ***T. rubrum*** and ***M. canis***.

On the other hand both lemon oil and orange oil had no fungistatic effect on both ***T. mentagrophytes*** and ***M. canis***.

Propolis with the concentration of (800 mg/100ml) had a very strong fungistatic effect on ***T. rubrum, T. mentagrophytes,*** and ***M. canis.***

Table 21 . Fungistatic and fungicidal effect of different essential oils and propolis against the tested dermatophytes fungi.

Essential oils (250 µL/100ml medium)	Dry weight (mg / 100ml medium)					
	T. rubrum	Activity	*T. mentagrophytes*	Activity	*M. canis*	Activity
Control	650		660		540	
Marjoram oil	238	fungistatic	77	fungistatic	125	fungistatic
Lemon oil	408	fungistatic	646	fungistatic	548	fungistatic
Orang oil	450	fungistatic	671	fungistatic	545	fungistatic
Mint oil	-	fungicidal	651	fungistatic	-	fungicidal
Chamomile oil	168	fungistatic	431	fungistatic	455	fungistatic
Flowers oil	192	fungistatic	597	fungistatic	484	fungistatic
Clove oil	-	fungicidal	-	fungicidal	-	fungicidal
Champhor oil	-	fungicidal	219	fungistatic	-	fungicidal
Garlic oil	-	fungicidal	201	fungistatic	109	fungistatic
Peach oil	153	fungistatic	-	fungicidal	148	fungistatic
Propolis	32	fungistatic	36	fungistatic	28	fungistatic

(-)= No growth

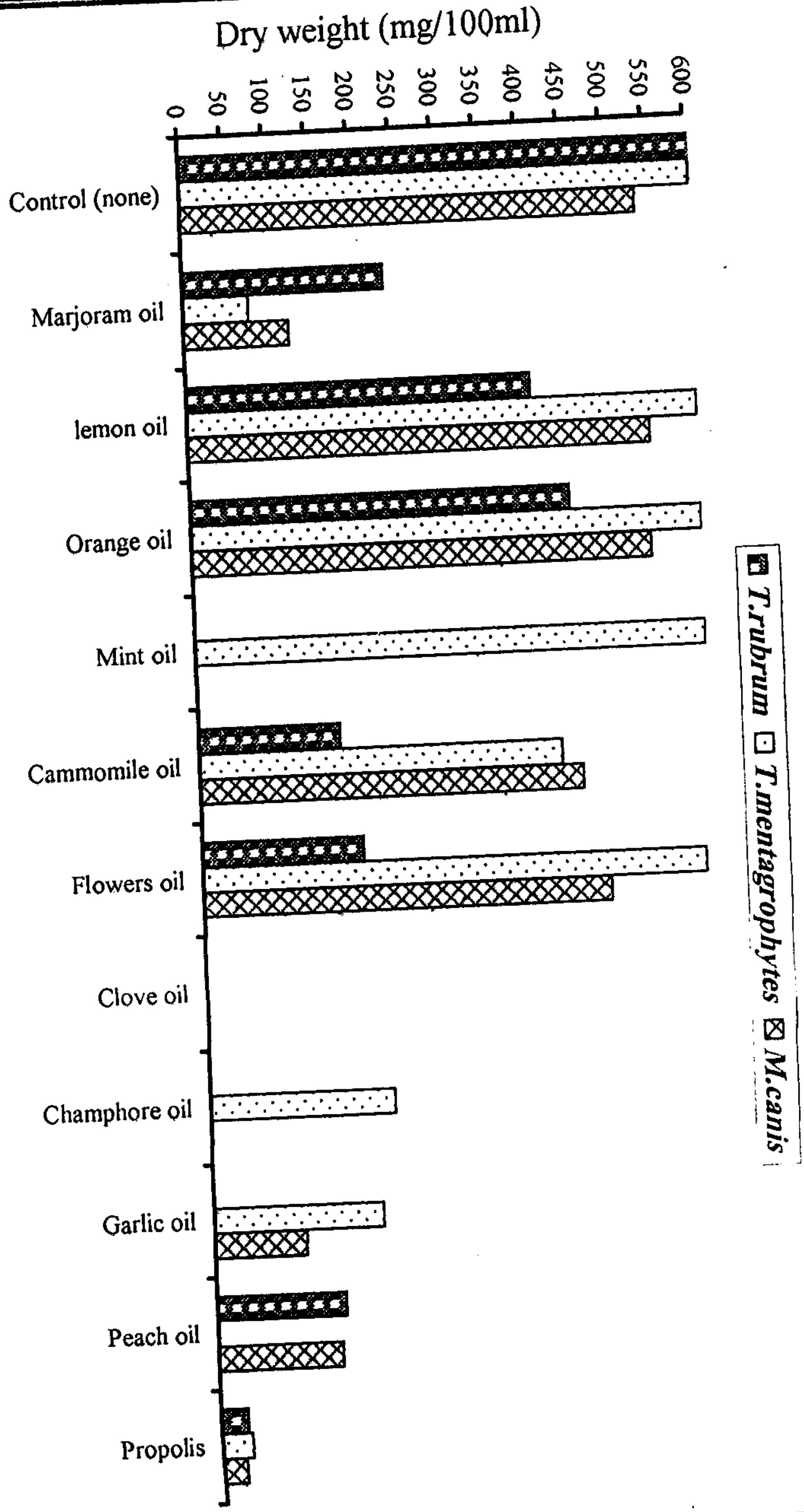


Fig. 22 . Fungistatic and fungicidal effect of different essential oils and propolis against tested dermatophyte fungi

4. Effect of different concentrations of commercial antifungal drugs (griseovulvin, Ketoconazole and nystatin) on the growth rate of tested dermatophyte fungi, compared with product of *S. kanamyceticus* EHE-68

The results in table (22) and figure (23) illustrate that the MFCs of griseofulvin, ketoconazole and nystatin against *T. rubrum, T. mentagrophytes* and *M. canis* were obtained under the best conditions (100% growth inhibition, 14 days of incubation and incubation temperature at 30°C). In general, the three drugs had high antifungal activity against the tested dermatophyte fungi. Over all, ketoconazole was the most active antifungal, showing the lowest MFC (MFC = 0.666 µl/100ml medium). Griseofulvin and nystatin were showed good antifungal activity (MFCs= 1.25µg and 107.2 µl /100ml medium, respectively).

Also, the crude antifungal product (ethanol extracted) by *St. kanamyceticus* EHE-68 gave 100% growth inhibition of *T. rubrum, T. mentagrophytes* and *M. canis* by MFC equal to 475 mg/100ml medium.

Table 22 . Comparison of the inhibitory effects of antifungal product (ethanol extracted) and antifungal drugs (griseofulvin, ketoconazole and nystatin) on *T. rubrum, T. mentagrophytes and M. canis*

Antifungal drugs with different concentrations	Inhibition percentages		
	T. rubrum	*T. mentagrophytes*	*M. canis*
Griseofulvin (µg/100ml)			
0.313	0%	0%	0%
0.625	40%	43%	48%
1.250	100%	100%	100%
2.500	100%	100%	100%
5.000	100%	100%	100%
Ketoconazole (µg/100ml)			
0.333	42%	40%	47%
0.666	100%	1005	100%
1.331	100%	100%	100%
2.665	100%	100%	100%
5.330	100%	100%	100%
Nystatin (µl/100ml)			
13.4	0%	0%	0%
26.8	0%	0%	0%
53.6	0%	0%	0%
80.4	42%	50%	53%
107.2	100%	100%	100%
Antifungal product (ethanol extracted) by *St.. kanamyceticus* (mg/100ml)			
120	31%	33%	35%
240	68%	70%	77%
370	83%	83%	86%
475	100%	100%	100%
960	100%	100%	100%

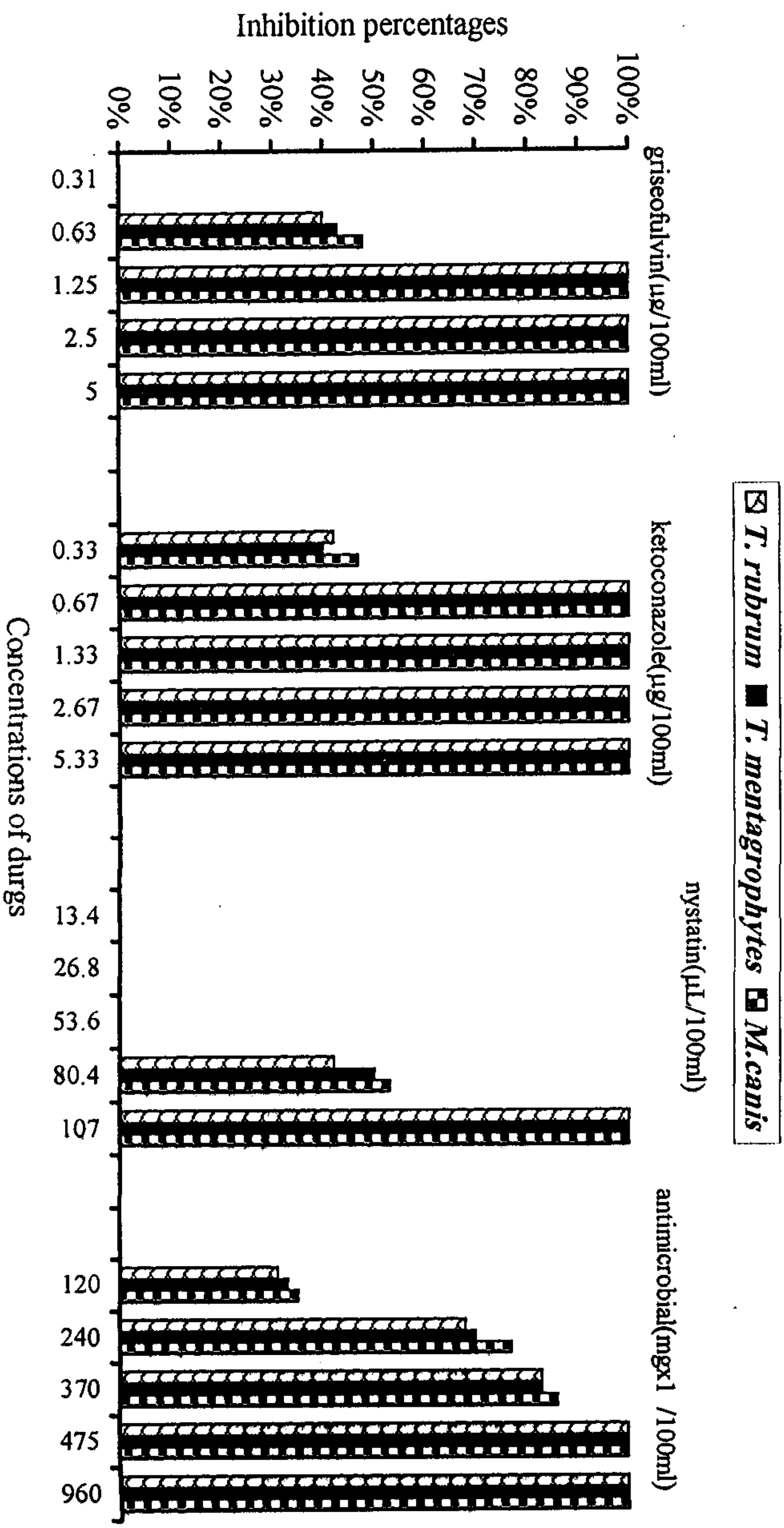


Fig. 23 . Comparison of the inhibitory effect of antifungal product (ethanol extracted) by *St. kanamyceticus* EHE-68 and antifungal drugs (grsieofulvin, ketoconazole and nystatin) on the tested dermatophyte fungi

5. Minimum fungalicidal concentration (MFCs) of some antifungal drugs, essential oils and antidermatophyte product by *St. kanamyceticus* EHE-68

Minimum fungalicidal concentrations (MFCs) were collected from previous results and illustrated in table (23) and fig (24) to comparison between MFCs of antifungal drugs used for treatment of fungal disease, essential oils used as antifungal and antidermatophyte product of tested ***St. kanamyceticus*** EHE-68 not concentrated and not purified. The results obtained illustrate that, the lowest MFCs of antifungal drugs was ketoconazol followed by griseofulvin and nystatin as antifungal drugs. Clove oil is the lowest MFCs of essential oils were used for all tested dermatophyte fungi. MFCs of propolis was determined at 80 mg/10 ml medium for all tested dermatophyte fungi. Non concentrated and non purified product of ***St. kanamyceticus*** EHE-68 was effected on growth of tested dermatophyte fungi but it was gained the highest MFC compared with antifungal drugs, essential oils and propolis. In future studying, I hope to concentrate and purify the product of ***St. kanamyceticus*** EHE-68 to obtain good results to use for treatment of dematophyte fungi diseases.

Table 23 : Minimum fungal concentrations(MFCs) of antifungal drugs, essential oils propolis and antifungal product of *S. kanamyceticus* EHE- 68

Antifungal tested	MFCs concentration of		
	T. rubrum	*T. mentagrophytes*	*M.canis*
Griseofulvin (µg /100ml)	1.25	1.25	1.25
Ketoconazole (µg /100ml)	0.666	0.666	0.666
Nystatin (µl /100ml)	107.2	107.2	107.2
Clove oil (µl /100ml)	31.25	31.25	31.25
Mint oil (µl /100ml)	125	-	31.25
Camomile (µl /100ml)	250	-	-
Champhor oil (µl /100ml)	250	-	125
Garlic oil (µl /100ml)	250	-	-
Peach oil (µl /100ml)	-	250	-
Marjoram oil (µl /100ml)	-	250	-
Propolis (mg /10ml)	80	80	80
Antifungal product (ethanol extracted) by *S.kanamyceticus* EHE-68 (mg /10 ml)	475	475	475

(-)= Not detected

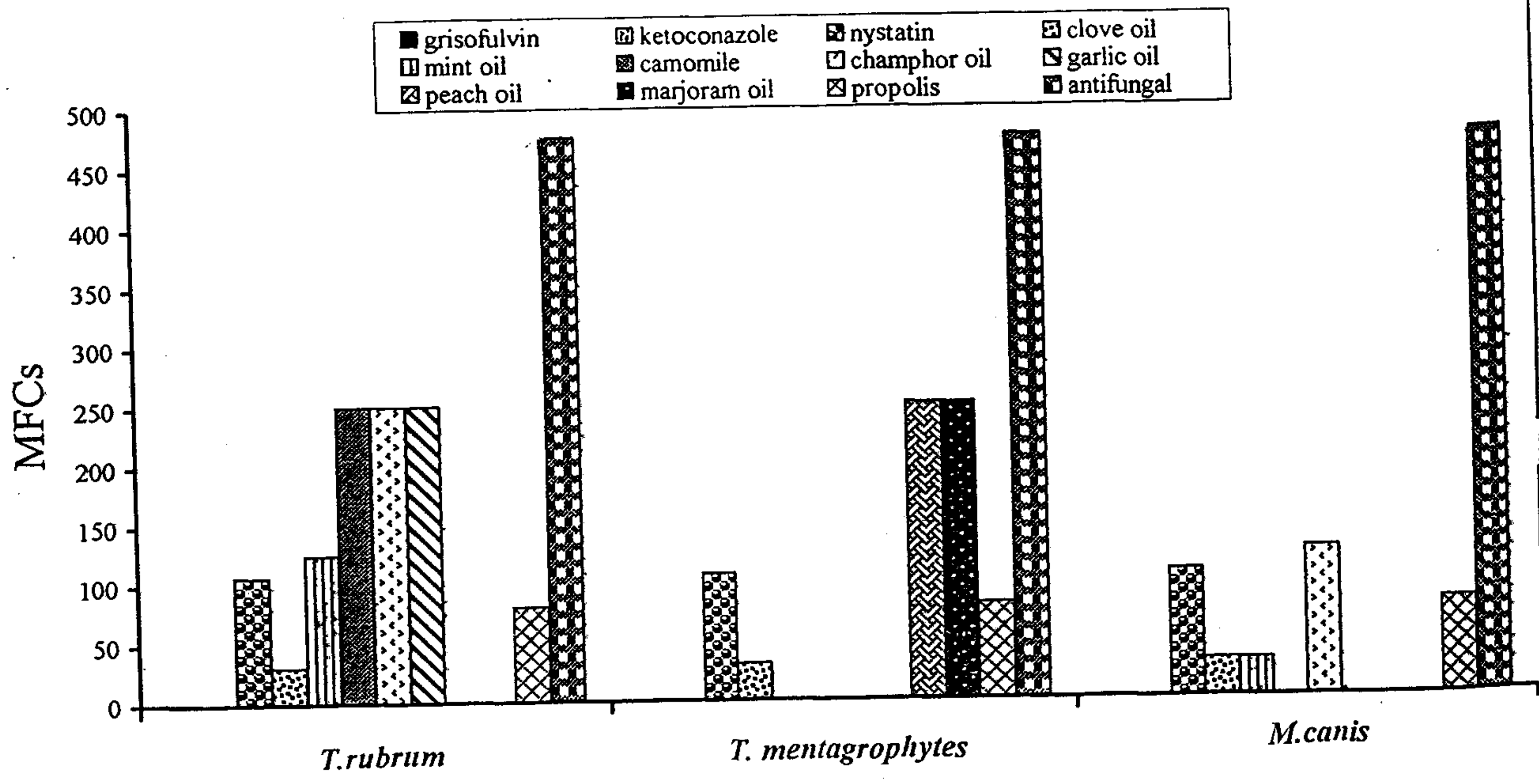


Fig. 24.. Comparison between MFCs of antifungal drugs, essential oils, propolis and antifungal product by *St. kanamyceticus* EHE-68